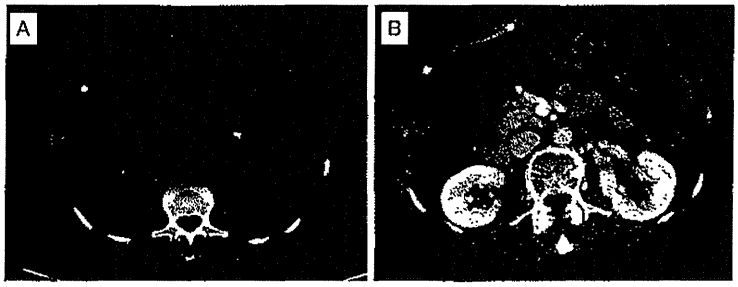
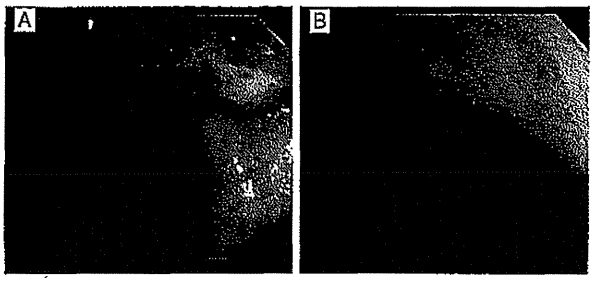


図❶ 症例1の腹部CT検査
A：治療前 B：治療後



図❷ 症例2の内視鏡像
A：治療前 B：治療後

2）症例2

40歳女性，原疾患はPSCであった．2001年に生体部分肝移植術を施行した．術後の免疫抑制薬は，タクロリムスとステロイド，MMFを使用した．移植後10年，下血を主訴に外来を受診した．下部消化管内視鏡で，肛門縁より15 cmの直腸に潰瘍性病変を認めた(図❷A)．生検をおこない，DLBCLと診断した．骨髄穿刺で病変の浸潤を認めず，胸腹部骨盤部のCT検査とFDG-PET CT検査をおこない，直腸病変と傍大動脈リンパ節の腫大を認めた．EBV-PCRは陰性であった．

治療は，免疫抑制薬の減量と化学療法をおこなった．タクロリムスの減量と，MMFを中止した．化学療法は，R-CHOP療法を合計8サイクルおこなった．有害事象は，白血球減少を5回，好中球減少を伴う感染を3回認めたものの，抗菌薬で軽快し，治療を完遂した．R-CHOP 4サイクル施行後，CT上病変の消失を認め，内視鏡下生検でも腫瘍細胞を認めず，CRと判定した(図❷B)．治療後の方針は症例1と同様とした．

3）症例の考察

当科で，成人（18歳以上）の生体肝移植例102例のうち，2例でPTLDの発症を認めた．発生頻度は2%であった．いずれも活動性EBV感染との関連性は示唆されず，移植後10年前後の経過で発症した（late-onset type）．病変は，Bリンパ球由来の悪性リンパ腫（monomorphic type）であり，病勢が強くリンパ節外性病変を認めた．原疾患は，ともにPSCであった．移植術後の経過中にPSC再燃と拒絶への危惧から免疫抑制薬を強めに維持していた．治療は，R-CHOP療法を施行し，2例とも大きな副作用なくCRを得られた．これらは，小児肝移植症例や欧米の多くの報告例とくらべ，わが国の成人生体肝移植例の特徴を示唆する可能性があると考えられた．

PTLD治癒後の免疫抑制薬の量には，議論の余地がある．EBV陰性PTLDの再発と免疫抑制薬の量については報告がない．当科では，免疫抑制薬を減量のままとし，拒絶反応を危惧しながら注意深く肝機能を評価する方針とした．また，治療中に肝生検を施行し，今後の拒絶反応の評価のコントロールとした．

◆おわりに

わが国における成人肝移植後のPTLDの報告はきわめて少ないが，今後，肝移植術後経過期間の長期化に伴い，PTLD症例は増加すると思われる．肝移植後PTLDは，その病因，リスクや治療経過についても不明な点が多い．PTLDの臨床的特徴は，施設間である程度異なることが報告されており，今後も症例数を重ね検討する必要がある．

文献

1) Steven HS *et al*：WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. IARC, Lyon, 2008, pp.343-349
2) Végso G *et al*：*Pathol Oncol Res* **17**：443, 2011
3) Kataoka K *et al*：*Leuk Lymphoma* **51**：1494, 2010
4) Kremers WK *et al*：*Am J Transplant* **6**：1017, 2006
5) Patel H *et al*：*Leuk Lymphoma* **48**：885, 2007

Shimada et al. World Journal of Surgical Oncology 2013, 11:52
http://www.wjso.com/content/11/1/52

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Clinicopathological characteristics and prognostic factors in young patients after hepatectomy for hepatocellular carcinoma

Shingo Shimada*, Toshiya Kamiyama, Hideki Yokoo, Kenji Wakayama, Yosuke Tsuruga, Tatsuhiko Kakisaka, Hirofumi Kamachi and Akinobu Taketomi

Abstract

Background: The aim of this study was to analyze the clinicopathological characteristics and the prognostic factors for survival and recurrence of young patients who had undergone hepatectomy for hepatocellular carcinoma.

Methods: Between 1990 and 2010, 31 patients aged 40 years or younger (younger patient group) among 811 consecutive patients with hepatocellular carcinoma who had undergone primary hepatectomy were analyzed with regard to patient factors, including liver function, tumor factors and operative factors. The clinicopathological characteristics of the younger patients were compared with those of patients over the age of 40 (older patient group). Then the prognostic factors of the younger patients were analyzed. Continuous variables were expressed as the means ± standard deviation and compared using the χ^2 test for categorical variables. Overall survival and recurrence-free survival rates were determined by the Kaplan-Meier method and analyzed by the log-rank test. The Cox proportional hazards model was used for multivariate analysis.

Results: In the younger patients, the rates of HBs-antigen-positivity, high alpha-fetoprotein, portal invasion, intrahepatic metastasis, large tumors, low indocyanin green retention rate at 15 minutes, and anatomical resection were significantly higher than the same measures in the older patients. The five-year overall survival rate of the young patients was 49.6%. The prognostic factors of survival were HCV-antibody-positivity and low albumin status. Prognostic factors of recurrence were multiple tumors and the presence of portal invasion.

Conclusions: In younger patients, survival appeared to be primarily affected by liver function, while recurrence was affected by tumor factors. Young patients with hepatocellular carcinoma should be aggressively treated with hepatectomy due to their good pre-surgical liver function.

Keywords: Hepatocellular carcinoma, Young, Hepatectomy, Clinicopathological characteristics, Prognostic factors

Background

Liver cancers are malignant tumors and are the third leading cause of cancer-related death; they are responsible for approximately 700,000 deaths per year [1]. Hepatocellular carcinoma (HCC) has a poor prognosis and accounts for 70 to 85% of primary liver cancers [2]. Generally, there are few opportunities for discovery of malignant tumors in younger patients, and thus they tend to present with a highly advanced malignancy at the time of diagnosis; nonetheless, younger patients can expect long-term survival. The definition of what constitutes a "young patient" differs between studies [3-12]. HCC is fairly rare in younger individuals, with an occurrence rate of only 0.6 to 2.7% in those under 40 years of age, according to Japanese reports [12-14]. In Asia and Africa, which are areas with prevalent hepatitis B virus (HBV), the frequency of HCC is higher than in Japan [4,8,9,11,15]; however, there are still few reports on independent prognostic factors in young patients with HCC.

In this study, we examined the prognostic clinicopathological features, as well as the prognostic factors for

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survival and recurrence, in young patients with HCC who had undergone hepatectomy.

Methods

Between January 1990 and May 2010, 811 consecutive patients with HCC underwent primary liver resection at the Gastroenterological Surgery I unit of Hokkaido University Hospital in Sapporo, Japan. Of these patients, 31 patients (3.8%) were 40 years old or younger, while 780 patients (96.2%) were over 40 years of age. For group stratification, the former patients were defined as the younger patient group, and the latter as the older patient group. This study was approved by the Hokkaido University Hospital Voluntary Clinical Study Committee and was performed according to the Helsinki Declaration guidelines. The clinicopathological characteristics and surgical data of the patients are shown in Table 1.

The indications for hepatic resection and the type of operative procedures were usually determined based on the patients' liver function reserve, that is, according to the results of the indocyanin green retention test at 15 minutes (ICGR15) [16]. Anatomical resection was performed on patients in whom the ICGR15 was lower than 25%. Anatomical resection was defined as a resection in which the lesions were completely removed anatomically on the basis of Couinauds' classification (segmentectomy, sectionectomy, and hemihepatectomy or more). Non-anatomical partial but complete resection was achieved in other cases. In all patients, surgery was performed at R0 or R1. When R0 and R1 resections were performed, the resection surfaces were found to be histologically or macroscopically free of HCC, respectively. Follow-up studies after liver resection were conducted at three-month intervals, which included physical, serological (liver function test, serum alpha-fetoprotein (AFP) level, and serum protein induced by vitamin K absence-II (PIVKA-II)), and radiological examinations (ultrasound sonography (US) and contrast-enhanced computed tomography (CT) scan or contrast-enhanced magnetic resonance imaging (MRI)). Recurrence was diagnosed on the basis of the results of contrast-enhanced CT and elevation of serum levels of AFP and/or PIVKA-II. Extrahepatic metastasis (lung, lymph node, adrenal gland, brain and bone) was diagnosed by contrast-enhanced chest and abdominal CT, contrast-enhanced head MRI and bone scintigram. The median follow-up period was 111 months (range, 5 to 249 months).

Statistical analysis

Continuous variables were expressed as the means ± standard deviation and compared using the χ^2 test for categorical variables. Overall survival (OS) and recurrence-free survival (RFS) were determined by the Kaplan-Meier

Table 1 Clinicopathological characteristics

	Young (age ≤40 years) n = 31	Old (age >40 years) n = 780	*P*
Epidemiology			
Sex: Male/Female	24/7 (77%/23%)	644/136 (83%/17%)	*NS*
HBs-Ag positive	26 (84%)	321 (41%)	<0.0001
HCV-Ab positive	1 (3%)	310 (40%)	<0.0001
Biochemical Factors			
Albumin ≥4.0 g/l	17 (55%)	411 (53%)	*NS*
Total bilirubin ≥0.8 mg/dl	17 (55%)	379 (49%)	*NS*
ICGR15 ≥15	3 (10%)	360 (46%)	0.0001
AFP ≥200 ng/ml	16 (52%)	210 (27%)	0.0026
Tumor Factors			
Number of tumors: 1	20 (65%)	522 (67%)	*NS*
2 to 3	6 (19%)	183 (23%)	
≥4	5 (16%)	75 (10%)	
Maximum size of tumors: <2 cm	4 (12%)	83 (11%)	0.0074
≥2 cm, <5 cm	7 (23%)	395 (50%)	
≥5 cm	20 (65%)	303 (39%)	
Macroscopic classification: simple nodular type	10 (32%)	408 (52%)	*NS*
simple nodular type with extranodular grow	10 (32%)	222 (28%)	
confluent multinodular type	8 (26%)	122 (16%)	
infiltrative type	0 (0%)	6 (1%)	
others	3 (10%)	22 (3%)	
Distant metastasis positive	2 (6%)	18 (2%)	*NS*
Surgical Factors			
Anatomical resection	29 (94%)	525 (67%)	0.0021
Histological Factors			
Differentiation: well	3 (10%)	114 (15%)	*NS*
moderate	13 (42%)	430 (55%)	
poor	14 (45%)	209 (27%)	
others	1 (3%)	27 (3%)	
vp:vp0	14 (45%)	569 (73%)	0.0026
vp1	9 (29%)	125 (16%)	
vp2,3,4	8 (26%)	86 (11%)	
im	16 (52%)	264 (34%)	0.0413
cirrhosis	9 (29%)	287 (37%)	*NS*

AFP, alpha-fetoprotein; HBs-Ag, HBs-antigen; HCV-Ab, HCV-antibody; ICGR15, indocyanin green retention rate at 15 minutes; im, microscopic intrahepatic metastasis; NS, non-significant; vp0, no tumor thrombus in the portal vein; vpl, tumor thrombus distal to the second branches of the portal vein; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor thrombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

method and analyzed by the log-rank test. The Cox proportional hazards model was used for multivariate analysis. Significance was defined as a *P*-value of <0.05. Statistical analyses were performed using Stat View 5.0 for Windows (SAS Institute, Cary, NC, USA).

Results

Clinicopathological characteristics and operative variables

Patient factors

The ratio of males to females (24:7) in the younger patient group was not significantly different from that of the older patient group. Patients with HBV markers accounted for most of the virus-associated cases: HBs-antigen (HBs-Ag)-positive, 26/31 (total number in the younger group) vs. 321/780 (total number in the older group); 84% vs. 41%; *P* <0.0001. Patients who were hepatitis C virus (HCV)-antibody (HCV-Ab)-positive were significantly fewer in number, that is, 1/31 vs. 310/780 (3% vs. 40%; *P* <0.0001) in the younger group. Although serum albumin and total bilirubin levels were not significantly different between the groups, patients with ICGR15 ≥15 were 3/31 vs. 360/780 (10% vs. 46%; *P* = 0.0001).

Tumor factors

The younger group had significantly higher AFP levels compared to the older group (*P* = 0.0026). Although the number of tumors did not differ significantly between the younger and older patients, there were significantly more cases with a maximum tumor size of ≥5 cm in the younger group (*P* = 0.0072). The mean maximum tumor diameter in the younger group in this study was 8.6 ± 7.3 cm. Neither macroscopic type nor extrahepatic metastasis was significantly different between the groups.

Operative variables

The rate of anatomical resections in the younger patients was significantly higher than that in the older patients.

Pathological factors

There were significant differences between groups in terms of microscopic tumor thrombus in the portal vein (*P* = 0.0026) and microscopic intrahepatic metastasis (*P* = 0.0413) (Table 1).

Causes of death and recurrence

Among the total 811 patients, 390 (48.1%) died. The mortality rates were 17/31 (54.8%) in the younger patient group and 373/780 (47.8%) in the older patient group. The causes of death, which did not differ significantly between groups, were as follows: HCC recurrence (n = 301; 77.2%; 16 in the younger patients vs. 285 in the older patients), liver failure (n = 36; 9.2%; 0 in the younger vs. 36 in the older patients), and other causes (n = 53; 13.6%; 1 in the younger vs. 52 in the older patients). In addition, two patients in the older group died of operative complications prior to 1995. No patients in the younger group died of operative complications.

In the younger group, 22 patients experienced a recurrence (71.0%). There were 17 (77.3%) liver tumor recurrences, with a median recurrence time of six months (1 to 27). Lung metastases occurred in 11 (50.0%) cases, with a median recurrence time of 12 months (1 to 42); bone metastases in 7 (31.8%) cases, with a median recurrence time of 23 months (6 to 60); brain metastases in 6 (27.3%) cases, with a median recurrence time of 20 months (10 to 61); lymph node metastases in 3 (13.6%) cases, with a median recurrence time of 12 months (12 to 56); and adrenal gland metastases in 3 (13.6%) cases, with a median recurrence time of 10 months (5 to 50).

Cumulative rates of patient survival and recurrence-free survival

The five-year OS rate of all 811 patients was 57.1%. The five-year OS rate and median survival time (MST) of the younger group were 49.6% and 40 months, respectively, whereas those of the older group were 57.7% and 79 months, respectively (Figure 1). The median RFS time of all 811 patients was 23 months, while that of the younger patients was 6 months, and that of the older patients was 25 months (Figure 2). Neither OS nor RFS were significantly different between the younger and older groups, although recurrence tended to occur earlier in the younger patients.

Factors related to long-term survival and disease-free survival after primary hepatectomy in the younger patient group

Table 2 shows those factors that were found by univariate analysis to influence OS and RFS in the younger

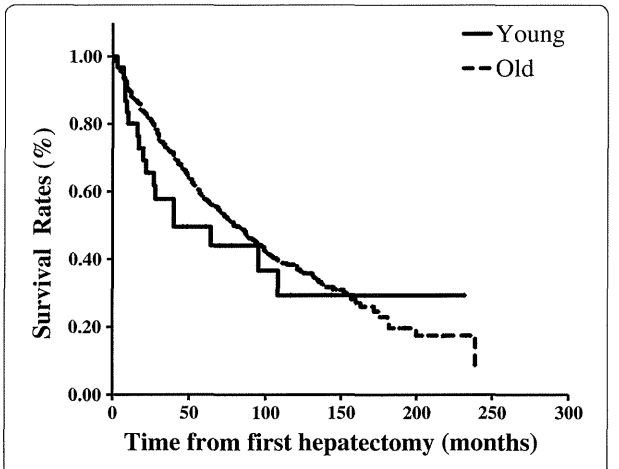

Figure 1 Overall survival curves of the younger and older patient groups after first hepatectomy.

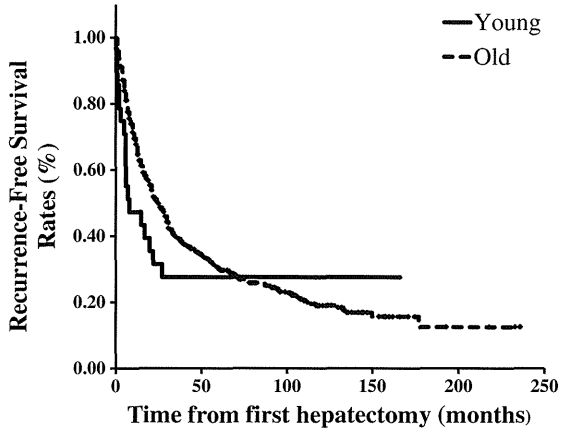

Figure 2 Recurrence-free survival curves of the younger and older patient groups after first hepatectomy.

Table 2 Univariate analyses of prognostic factors of survival and recurrence in the younger group

	Survival	Recurrence
	P	*P*
Epidemiology		
Sex: Male	*NS*	*NS*
HBs-Ag positive	*NS*	*NS*
HCV-Ab positive	0.0172	*NS*
Biochemical Factors		
Albumin <4.0 g/l	0.0088	*NS*
Total bilirubin ≥0.8 mg/dl	*NS*	*Ns*
ICGR15 ≥15	*NS*	*NS*
AFP ≥200 ng/ml	*NS*	*NS*
Tumor Factors		
Number of tumors: multiple	*NS*	0.0199
Maximum size of tumor: ≥5 cm	0.0034	0.0006
Macroscopic classification: except for simple nodular type	*NS*	*NS*
Distant metastasis positive	*NS*	-
Surgical Factors		
Non-anatomical resection	*NS*	*NS*
Histological Factors		
Differentiation: poor	*NS*	0.0395
vp2, 3, 4	0.0108	0.0020
im	0.0058	0.0053
cirrhosis	0.0446	*NS*

AFP, alpha-fetoprotein; HBs-Ag, HBs-antigen; HCV-Ab, HCV-antibody; ICGR15, indocyanin green retention rate at 15 minutes; im, microscopic intrahepatic metastasis; NS, non-significant; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor thrombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

group. The univariate analysis revealed that OS was significantly related to being HCV-Ab-positive, having a serum albumin level of <4.0 g/l and a maximum tumor size of ≥5 cm, the presence of tumor thrombus in the second and first branches and trunk or opposite side branch of the portal vein (vp2, 3, 4), microscopic intrahepatic metastasis, and histological liver cirrhosis of non-cancerous liver.

Univariate analysis showed that RFS was significantly related to multiple tumors, maximum tumor size of ≥5 cm, poor differentiation, the presence of tumor thrombus above vp2 and microscopic intrahepatic metastasis. Multivariate analysis showed HCV-Ab-positive status and serum albumin levels of <4.0 g/l to be independent predictive factors for OS, and multiple tumors and vp2, 3, 4 were independent predictive factors for RFS in the younger group of patients (Tables 3 and 4).

Discussion

In this study, the younger patients with HCC who underwent hepatectomy were more likely than the older patients to be HBV-positive, to have large tumors with portal invasion and to have high AFP, although they also retained better liver function than the older patients. Despite the significant difference in tumor progression, neither OS nor RFS were significantly different between the two groups, although recurrence tended to occur earlier in the younger patients. Multivariate analysis showed HCV-Ab-positive status and serum albumin levels of <4.0 g/l to be independent predictive factors for OS, and multiple tumors and vp2, 3, 4 were independent predictive factors for RFS in the younger patients. Therefore, young patients with hepatocellular carcinoma should be aggressively treated with hepatectomy due to their good pre-surgical liver function.

In the younger group of patients, HCV-Ab-positive status and low serum albumin levels were the liver-function-related factors that were found to be significantly unfavorable in terms of OS, while multiple tumors

Table 3 Multivariate analyses of prognostic factors of survival in the younger group

Risk factor	*P*-value	Hazard ratio	95% CI
HCV-Ab positive	0.0196	59.816	1.927 to 1856.714
Albumin <4.0 g/l	0.0296	6.665	1.207 to 36.813
Maximum size of tumor: ≥5 cm	*NS*	0.381	0.025 to 5.697
vp2, 3, 4	*NS*	2.313	0.420 to 12.738
im	*NS*	14.563	0.951 to 222.939
cirrhosis	*NS*	1.037	0.149 to 7.200

CI, confidence interval; HCV-Ab, HCV-antibody, im, microscopic intrahepatic metastasis; NS, non-signficant; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor rhombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

Table 4 Multivariate analyses of prognostic factors of recurrence in the younger group

Risk factor	*P*-value	Hazard ratio	95% CI
Number of tumor: multiple	0.0415	51.312	1.163 to 2264.565
Maximum size of tumor: ≥5 cm	*NS*	3.210	0.353 to 29.152
Differentiation: poor	*NS*	2.796	0.450 to 17.043
vp2, 3, 4	0.0253	13.517	1.380 to 132.442
im	*NS*	0.137	0.005 to 3.541

CI, confidence interval; im, microscopic intrahepatic metastasis; NS, non-significant; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor thrombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

and vp2, 3, 4 were the tumor-related factors that were significantly unfavorable in terms of RFS; moreover, these findings were obtained by both univariate and multivariate analyses. Although most of the younger patients had advanced tumors, no differences were found between the younger and older patients in terms of OS. These results indicate that aggressive and curative liver resection should be performed for young patients with HCC, because most young patients retain good presurgical liver function.

The definition of who should be classified as a "young patient" with HCC remains controversial. In the literature, the definition of a young patient with HCC has tended to be a patient aged 40 years or younger [4,8,10-12,14]. Cases of HCC in such patients are comparatively rare, for example, HCC occurs in only 0.6 to 2.7% of this age group in Japanese reports [12-14]. In other countries, the reported rates of HCC in this age range are as follows: 8.6% (40 years and younger) in Singapore [11], 10.9% (under 40 years) in Taiwan [8] and 6.5% (40 years and younger) in Hong Kong [4]. Thus most of the existing reports have been from Asia, and they show a difference in frequency among regions. There appear to be many young patients in Asia with HCC who are HBV-positive; HBV is an underlying disease of HCC in young patients, and many carriers live in Asia [17].

Many young patients with HCC have HBs-Ag, that is, up to 71.4 to 100% [3-5,7-11,14]. Meanwhile, cases of HCV-Ab-positivity plus HCC among younger patients are reported at rates of 0 to 10% [4,5,7-10,12,14], which is much lower than the range for older patients. Rates of Child-Pugh A are 69.1 to 92.3% among younger patients [4-6,8-12], which is higher than the range in older patients. It has been reported that histological hepatitis or cirrhosis of non-cancerous liver is significantly less common in younger hepatectomy patients than in older hepatectomy patients among cases with HCC [3,4,12]. Though HCC is generally found by medical examination or follow-up of liver function, in most young patients, HCC is found by symptoms such as pain and/or palpation of an abdominal mass [11,14,18,19]. Accordingly, members of the younger patient group in this study had larger tumors than the older patient group.

This study revealed that the rate of cases related to HBV was 93.5%, and the rate of HBs-Ag-positive cases was 87.0%. The MST of the younger group was 40 months, and the five-year OS rate was 49.6%. These results did not differ significantly from the previously reported MST and five-year OS rates of 27.8 to 52.5 months and 30.5 to 54.8%, respectively, among cases of liver resection for HCC across all ages [20,21]. Therefore, it appears likely that aggressive and curative liver resection contributes to prolonged prognosis.

In regard to tumor factors, several studies have reported that more young than old patients have high AFP levels, that is, the rates of cases in which AFP is equal to or exceeds a value of 400 ng/ml range from 52.6 to 82.0% [3,7,9-11,14], and rates for an AFP of ≥10,000 ng/ml range from 31.6 to 60.0% [3,10,11,14]. In addition, younger patients tend to have larger tumors than older patients, with the maximum diameter of tumors being 6.9 to 12.7 cm in younger patients [3,4,7,10,12,14]. Cases showing portal invasion count for 45.0 to 100% [10-12,14] of younger HCC patients. In the present study, the younger patient group had higher AFP levels and larger tumors, was more likely to have portal invasion and showed better liver function than the older group, as has been reported elsewhere [3,7,10-12,14]. It has also been reported that cases with high AFP levels have a poor prognosis due to a correlation between tumor size and AFP [22].

As regards prognostic factors, Chen *et al.* reported that hepatectomy was a significant favorable prognostic factor among HCC patients aged 40 years and younger [8]. As regards other prognostic factors, AFP [8,11], portal invasion [8,11] and reserved liver function [8,11,12] have been reported, although these remain controversial. In this study, prognostic factors related to OS were HCV-Ab-positive status and low serum albumin levels, and prognostic factors related to RFS were the number of tumors and vp2, 3, 4. It has been suggested that liver function preservation primarily influences survival, and tumor factors influence recurrence. Furthermore, while the time to recurrence in the younger patients was shorter than that in the older patients, the RFS of the younger group tended to overtake that of the older group in the long term. The recurrence rate was 71%, and the site of recurrence was almost always the liver. This rate was comparable to those of other reports, which ranged from 60.2 to 78.2% across all ages [20]. The results to date suggest that aggressive treatments, including re-hepatectomy for recurrence, contribute to an improvement in the long-term prognosis.

Moreover, in order to improve prognosis, we should take care to perform aggressive resections, and should also make note of cases with a background of potentially liver-affecting hepatitis B. Chuma *et al.* reported that the quantity of HBV-DNA and non-treatment for HBV were risk factors for a recurrence of HCC [23]. Li *et al.* reported that one-year and two-year RFS rates were 23.3% vs. 8.3%, and 2.3% vs. 0%, respectively, in a treatment group receiving lamivudine for HCC due to concurrent hepatitis B vs. a control group [24]. Therefore, viral treatments in combination with cancer treatments, including resection, are important to consider.

There have been few reports on liver transplantation for young patients with HCC. The reason for this lack of information is likely to be that younger patients have relatively larger tumors and, therefore, they tend to have tumors exceeding the Milan criteria. Ismail *et al.* reported that the outcomes of liver transplantation were better than those of liver resection among patients with HCC who were aged 2 to 27 years, namely, the OS rates were 72% vs. 40%, and the RFS rates were 91% vs. 30% [25]. It was also reported that primary liver transplantation for children with HCC without extrahepatic lesions has a good outcome, even if the tumors exceed the Milan criteria [26]. An accumulation of future cases is expected.

As noted above, many young HCC patients present with advanced tumors and unfavorable prognostic factors. In a study on 16 patients who received liver transplantation for HCC and who had low differentiation and vascular invasion beyond the Milan criteria, Saab *et al.* reported that those receiving sorafenib (n = 8) had one-year OS rates and RFS rates of 87.5% and 85.7%, versus 62.5% and 57.1% for the control group (n = 8) [27]. It is expected that supportive treatment with molecular target medicine after liver resection or transplantation could contribute to a prolonged prognosis.

Conclusions

In our younger patients with HCC, survival appeared to be mainly affected by liver function while recurrence was mainly affected by tumor factors. Young patients with HCC should be offered aggressive hepatectomy due to their relatively preserved liver function.

Abbreviations

AFP: Alpha-fetoprotein; CT: Computed tomography; HBV: Hepatitis B virus; HBs-Ag: HBs-antigen; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HCV-Ab: Hepatitis C virus-antibody; ICGR15: Indocyanin green retention test at 15 minutes; MRI: Magnetic resonance imaging; MST: Median survival time; OS: Overall survival; PIVKA-II: Protein induced by vitamin K absence-II; RFS: Recurrence-free survival; US: Ultrasound sonography; vp2: Tumor thrombus in the second branches of the portal vein; vp3: Tumor thrombus in the first branch of the portal vein; vp4: Tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

Competing interests

All of the authors declare that they have no competing interests.

Authors' contributions

SS carried out the analysis of data and wrote the manuscript. TK and AT gave comments and revised the manuscript. HY, KW, YT, TK and HK made the database of patients. All authors read and approved the final manuscript.

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: **Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.** *Int J Cancer* 2010, **127**:2893–2917.
2. Ahmed F, Perz JF, Kwong S, Jamison PM, Friedman C, Bell BP: **National trends and disparities in the incidence of hepatocellular carcinoma,1998-2003.** *Prev Chronic Dis* 2008, **5**:A74.
3. Furuta T, Kanematsu T, Matsumata T, Shirabe K, Yamagata M, Utsunomiya T, Sugimachi K: **Clinicopathologic features of hepatocellular carcinoma in young patients.** *Cancer* 1990, **66**:2395–2398.
4. Lam CM, Chan AO, Ho P, Ng IO, Lo CM, Liu CL, Poon RT, Fan ST: **Different presentation of hepatitis B-related hepatocellular carcinoma in a cohort of 1863 young and old patients - implications for screening.** *Aliment Pharmacol Ther* 2004, **19**:771–777.
5. Sezaki H, Kobayashi M, Hosaka T, Someya T, Akuta N, Suzuki F, Tsubota A, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Matsuda M, Takagi K, Sato J, Kumada H: **Hepatocellular carcinoma in noncirrhotic young adult patients with chronic hepatitis B viral infection.** *J Gastroenterol* 2004, **39**:550–556.
6. Klein WM, Molmenti EP, Colombani PM, Grover DS, Schwarz KB, Boitnott J, Torbenson MS: **Primary liver carcinoma arising in people younger than 30 years.** *Am J Clin Pathol* 2005, **124**:512–518.
7. Kim JH, Choi MS, Lee H, Kim do Y, Lee JH, Koh KC, Yoo BC, Paik SW, Rhee JC: **Clinical features and prognosis of hepatocellular carcinoma in young patients from a hepatitis B-endemic area.** *J Gastroenterol Hepatol* 2006, **21**:588–594.
8. Chen CH, Chang TT, Cheng KS, Su WW, Yang SS, Lin HH, Wu SS, Lee CM, Changchien CS, Chen CJ, Sheu JC, Chen DS, Lu SN: **Do young hepatocellular carcinoma patients have worse prognosis? The paradox of age as a prognostic factor in the survival of hepatocellular carcinoma patients.** *Liver Int* 2006, **26**:766–773.
9. Cho SJ, Yoon JH, Hwang SS, Lee HS: **Do young hepatocellular carcinoma patients with relatively good liver function have poorer outcomes than elderly patients?** *J Gastroenterol Hepatol* 2007, **22**:1226–1231.
10. Yamazaki Y, Kakizaki S, Sohara N, Sato K, Takagi H, Arai H, Abe T, Katakai K, Kojima A, Matsuzaki Y, Mori M: **Hepatocellular carcinoma in young adults: the clinical characteristics, prognosis, and findings of a patient survival analysis.** *Dig Dis Sci* 2007, **52**:1103–1107.
11. Chang PE, Ong WC, Lui HF, Tan CK: **Is the prognosis of young patients with hepatocellular carcinoma poorer than the prognosis of older patients? A comparative analysis of clinical characteristics, prognostic features, and survival outcome.** *J Gastroenterol* 2008, **43**:881–888.
12. Takeishi K, Shirabe K, Muto J, Toshima T, Taketomi A, Maehara Y: **Clinicopathological features and outcomes of young patients with hepatocellular carcinoma after hepatectomy.** *World J Surg* 2011, **35**:1063–1071.
13. Tanioka H, Omagari K, Kato Y, Nakata K, Kusumoto Y, Mori I, Furukawa R, Tajima H, Koga M, Yano M, Kohno S: **Present status of hepatitis virus-associated hepatocellular carcinoma in Nagasaki Prefecture, Japan: a cross-sectional study of 1019 patients.** *J Infect Chemother* 2002, **8**:64–69.
14. Aramaki M, Kawano K, Sasaki A, Ohno T, Tahara K, Kai S, Iwashita Y, Kitano S: **Hepatocellular carcinoma in young adults.** *Hepatogastroenterology* 2005, **52**:1795–1797.
15. Kew MC: **Clinical, pathologic, and etiologic heterogeneity in hepatocellular carcinoma: evidence from southern Africa.** *Hepatology* 1981, **1**:366–369.

16. Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Yamashita K, Taniguchi M, Shimamura T, Matsushita M, Todo S: **Perioperative management of hepatic resection toward zero mortality and morbidity: analysis of 793 consecutive cases in a single institution.** *J Am Coll Surg* 2010, **211**:443–449.
17. Dan YY, Aung MO, Lim SG: **The economics of treating chronic hepatitis B in Asia.** *Hepatol Int* 2008, **2**:284–295.
18. Ni YH, Chang MH, Hsu HY, Hsu HC, Chen CC, Chen WJ, Lee CY: **Hepatocellular carcinoma in childhood. Clinical manifestations and prognosis.** *Cancer* 1991, **68**:1737–1741.
19. Hernandez-Castillo E, Mondragon-Sanchez R, Garduno-Lopez AL, Gomez-Gomez E, Ruiz-Molina JM, Onate-Ocana LF, Bernal-Maldonado R: **Hepatocellular carcinoma in the youth. A comparative analysis with hepatocellular carcinoma in adulthood.** *Hepatogastroenterology* 2005, **52**:903–907.
20. Fan ST, Lo CM, Poon RT, Yeung C, Liu CL, Yuen WK, Lam CM, Ng KK, Chan SC: **Continuous improvement of survival outcomes of resection of hepatocellular carcinoma: a 20-year experience.** *Ann Surg* 2011, **253**:745–758.
21. Yang T, Lin C, Zhai J, Shi S, Zhu M, Zhu N, Lu JH, Yang GS, Wu MC: **Surgical resection for advanced hepatocellular carcinoma according to Barcelona Clinic Liver Cancer (BCLC) staging.** *J Cancer Res Clin Oncol* 2012, **138**:1121–1129.
22. Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC: **High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations.** *Int J Cancer* 2004, **112**:44–50.
23. Chuma M, Hige S, Kamiyama T, Meguro T, Nagasaka A, Nakanishi K, Yamamoto Y, Nakanishi M, Kohara T, Sho T, Yamamoto K, Horimoto H, Kobayashi T, Yokoo H, Matsushita M, Todo S, Asaka M: **The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma.** *J Gastroenterol* 2009, **44**:991–999.
24. Li N, Lai EC, Shi J, Guo WX, Xue J, Huang B, Lau WY, Wu MC, Cheng SQ: **A comparative study of antiviral therapy after resection of hepatocellular carcinoma in the immune-active phase of hepatitis B virus infection.** *Ann Surg Oncol* 2010, **17**:179–185.
25. Ismail H, Broniszczak D, Kalicinski P, Markiewicz-Kijewska M, Teisseyre J, Stefanowicz M, Szymczak M, Dembowska-Baginska B, Kluge P, Perek D, Kosciesza A, Dzik E, Lembas A, Teisserye M: **Liver transplantation in children with hepatocellular carcinoma. Do Milan criteria apply to pediatric patients?** *Pediatr Transplant* 2009, **13**:682–692.
26. Romano F, Stroppa P, Bravi M, Casotti V, Lucianetti A, Guizzetti M, Sonzogni A, Colledan M, D'Antiga L: **Favorable outcome of primary liver transplantation in children with cirrhosis and hepatocellular carcinoma.** *Pediatr Transplant* 2011, **15**:573–579.
27. Saab S, McTigue M, Finn RS, Busuttil RW: **Sorafenib as adjuvant therapy for high-risk hepatocellular carcinoma in liver transplant recipients: feasibility and efficacy.** *Exp Clin Transplant* 2010, **8**:307–313.

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CASE REPORT

Hepatic osteodystrophy complicated with bone fracture in early infants with biliary atresia

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Abstract

Biliary atresia (BA) is one of the major hepatobiliary abnormalities in infants and one of the causes of hepatic osteodystrophy. Bone disease may be caused by the malabsorption of calcium and magnesium by vitamin D in hepatobiliary diseases in which bile flow into the intestines is deficient or absent. Bone fracture before Kasai hepatic portoenterostomy or within one month after the procedure in an infant with BA is very rare. We herein report two infants: one infant with BA who initially presented with a bone fracture before Kasai hepatic portoenterostomy, and the other at 4 wk after Kasai hepatic portoenterostomy, and also provide a review of the literature. Moreover, we conclude that clinicians should consider BA in infants with bone fracture during early infancy.

Key words: Biliary atresia; Bone fractur; Hepatic osteodystrophy; Kasai hepatic portoenterostomy; Vitamin D deficiency

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INTRODUCTION

Clinical findings in children with biliary atresia (BA) characteristically include jaundice and acholic stools at 1 or 2 mo after birth[1]. Osteodystrophy is a well-recognized complication of chronic liver disease. BA is one of the major hepatobiliary abnormalities in infants and one of the causes of hepatic osteodystrophy[1].

Vitamin D is hydroxylated at the carbon 25 position to form 25-hydroxy-vitamin D (25-OH-D)[2]. This occurs primarily in the liver[2]. Bile is important for the intestinal absorption of calcium and magnesium because it is necessary for the absorption of vitamin D[1].

In chronic liver disease, particularly where there is chronic cholestasis, generalized skeletal demineralization or rachitic change is seen[3]. Multiple spontaneous fractures of both the ribs and long bones have been reported in such infants. Furthermore, bone fractures are sometimes noted in patients with BA in the end-stage before liver transplantation[4]. However, bone fracture before Kasai hepatic portoenterostomy and within one month after the procedure in infants with BA is very rare.

We report two infants: firstly, a patient with BA who initially presented with bone fracture before Kasai he-

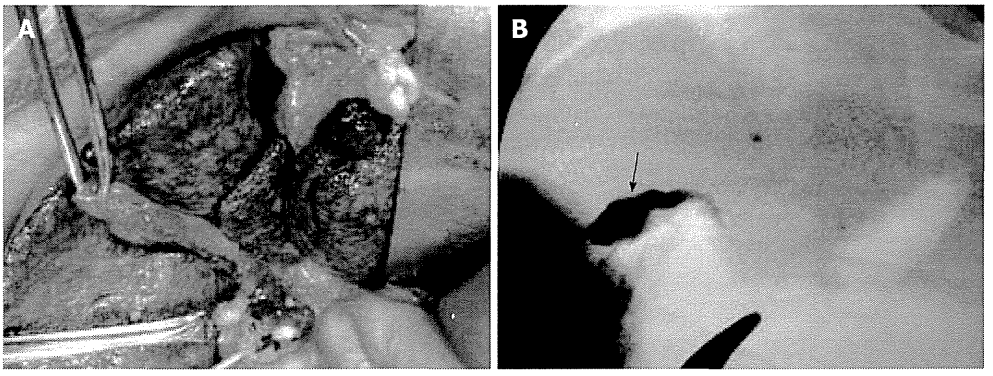

Figure 1 Intraoprative and imaging features. A: On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis; B: Intraoperative cholangiography revealed a patent gallbladder (arrow) and no patency of the extrahepatic bile duct.

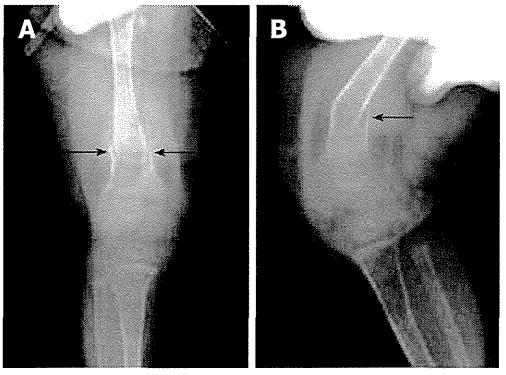

Figure 2 Plain skeletal radiographic features at the 7 d after hepaticojejunostomy in the case 1. Anteroposterior (A) and lateral (B) plain radiographs showing a displaced fracture (arrows) of the right distal femur.

patic portoenterostomy, and secondly, a patient with the onset of bone fracture within one month after Kasai hepatic portoenterostomy, and also provide a review of the literature.

CASE REPORT

Case 1

A girl was born vaginally at 39 wk gestation, weighing 2522 g. She presented with neither jaundice nor acholic stools. The infant was fed human milk. She was well nourished but was observed to have jaundice at a medical check-up at 1 mo of age. Abdominal ultrasonography (US) and computed tomography showed a sufficiently large gallbladder. Total and direct bilirubin (DB) decreased gradually at the follow-up checks. The patient presented with acholic stools and increased jaundice at the age of 5 mo, and was subsequently admitted to our institution for further examinations. Laboratory studies upon admission revealed the following: asparate aminotransferase (AST) 337 IU/L (normal range), alanine aminotransferase (ALT) 241 IU/L (normal range), total bilirubin (TB) 11.3 mg/dL, DB 7.4 mg/dL, alkaline phosphatase (ALP) 5,547 IU/L (normal range), γ-glutamyl transpeptidase (γ-GTP) 457 IU/L (normal range), choline esterase 192 IU/L (normal range), and serum calcium 8.1 mg/dL (normal range). There was severe jaundice noted at the conjunctiva. The findings on abdominal US were unevenness on the liver surface and an atrophic gallbladder which did not contract after the feeding of milk. Magnetic resonance cholangio-pancreatography (MRCP) revealed dilatation of neither the common bile duct nor intrahepatic bile duct. Therefore, BA was suspected based on these findings, and the infant underwent an exploratory laparotomy at 182 d of age. The patient started oral vitamin D at 173 d of age.

On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis (Figure 1A). Intraoperative cholangiography revealed a patent gallbladder and no patency of the extrahepatic bile duct (Figure 1B). The macroscopic findings showed that the bilateral hepatic ducts and extrahepatic bile duct consisted of only remnants. The infant was diagnosed as BA (II bɪγ)[5] based on cholangiographic and macroscopic findings. The remnants were totally removed en block and a Roux-en-Y hepaticojejunostomy was performed with a Roux loop of 60 cm applied antecolically. Microscopic findings of the liver biopsy specimen were pre-cirrhotic.

The patient could not move her right leg 1 d before the laparotomy, and a plain skeletal radiograph of the femur was performed 7 d after the HJ, when the general condition of the patient was stable. A displaced fracture of the right distal femur was shown by the plain radiograph (Figure 2A and B). Hepatic osteodystrophy was suspected based on the fact that there was no history of femur trauma and the patient suffered from chronic cholestasis. Child abuse by the family was not considered from the situation. Callus formation was seen 8 d after the application of an immobilizing plaster bandage (Figure 3A). The plaster bandage was removed after 20 d and the fracture of the right femur was cured at 6 mo post fracture (Figure 3B and C). The patient coughed up blood due to the perforation of esophageal varices and underwent a living-related liver transplantation at 10 mo of age. The postoperative course of living-related liver transplantation was uneventful and she is currently well at 4 years of age.

Case 2

A girl was born vaginally at 36 wk gestation, weighing 2310 g. She presented with neither jaundice nor acholic stools. She was well nourished but was observed to have

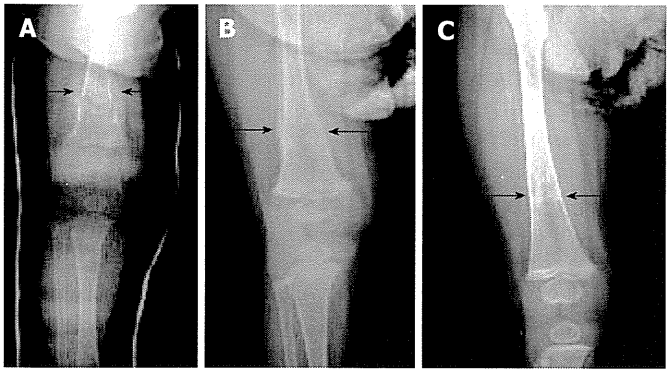

Figure 3 Plain skeletal radiographic features at the 8 d after the application of an immobilizing plaster bandage for the femur fracture in the case 1. Callus formation (arrows) was seen 8 d after the application of an immobilizing plaster bandage (A) in case 1. The plaster bandage was removed after 20 d (B) and the fracture of the right femur was cured 6 mo post-fracture (C).

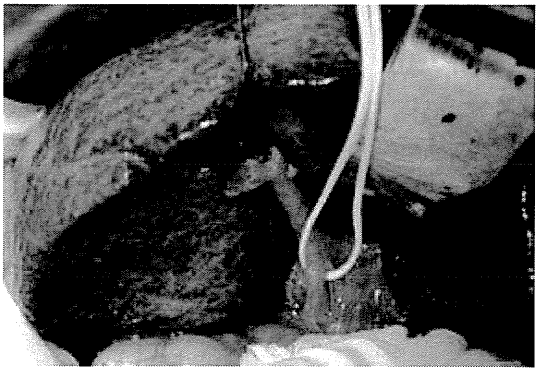

Figure 4 Intraoprative features. On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis.

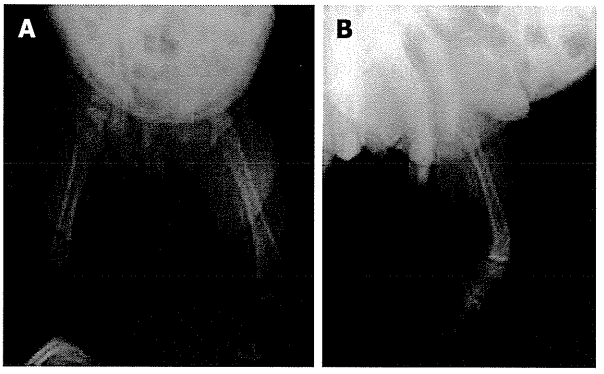

Figure 5 Plain skeletal radiographic features at the 28 d after hepaticojejunostomy in the case 2. Anteroposterior (A) and lateral (B) plain radiographs showing a displaced fracture (arrows) of the left distal femur.

jaundice at a medical check-up at 1 mo of age. The patient presented with acholic stools and increased jaundice at the age of 3 mo at a medical check-up, and was consequently admitted to our institution for further examinations. Laboratory studies upon admission revealed the following: AST 573 IU/L, ALT 377 IU/L, TB 6.6 mg/dL, DB 4.4 mg/dL, ALP 2248 IU/L, γ-GTP 666 IU/L, choline esterase 181 IU/L, and serum calcium 9.2 mg/dL. The findings on abdominal US and MRCP were just as same as those of the case 1. Therefore, BA was suspected, and the infant underwent an exploratory laparotomy at 113 d of age. The patient started oral vitamin D at 3 mo of age.

On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis (Figure 4). Intraoperative cholangiography revealed a patent gallbladder and no patency of the extrahepatic bile duct. The infant was diagnosed as BA (Ⅱ b1γ)[5] based on cholangiographic and macroscopic findings. The remnants were totally removed en block and a Roux-en-Y hepaticojejunostomy was performed with a Roux loop of 60 cm applied antecolically. Microscopic findings of the liver biopsy specimen were cirrhotic.

The patient could not move her left leg at 28 d post-laparotomy. A displaced fracture of the left distal femur was shown by plain skeletal radiograph (Figure 5A and B). Hepatic osteodystrophy was suspected based on the fact that there was no history of femur trauma and the patient suffered from chronic cholestasis. Child abuse by the family was not considered from the situation. Callus formation was seen 14 d after the application of an immobilizing plaster bandage. The plaster bandage was removed after 20 d and the fracture of the left femur was cured at 6 mo after post-fracture. Jaundice has been resolved and she is currently well at 11 mo of age.

DISCUSSION

BA is a rare disease with an incidence of approximately 1:10 000 live births in Japan and the Far East[6]. The most frequent symptom is prolonged jaundice. Several reports have shown that osteodystrophy was associated with severe chronic liver disease despite the administration of vitamin and mineral supplements[1]. Argao *et al*[7] suggested that the bone mineral content of patients with hepatic osteodystrophy did not improve despite successful normalization of the serum 25-OH vitamin D concentration by enhancing vitamin D absorption from the gastrointestinal tract. Chongsrisawat *et al*[8] reported that osteoporosis was recognized in up to 80% of a group of jaundiced BA patients in comparison with only 13.6% in a non-jaundiced group.

In BA, metabolic disturbance results from impairment of the passage of bile salts into the alimentary canal. As a consequence, the inadequate emulsification of fat results

in the incomplete absorption of vitamin D. Vitamin D is hydroxylated to 25-OH-D in the liver[2]. Additionally, over the course of the disease, liver cirrhosis develops and the hydroxylation of vitamin D is impaired. Vitamin D and hence calcium absorption are thus diminished. 25-OH-D is thought to be converted to more active forms, 125- or 2125-dehydro-OH-D. Rickets and osteoporosis were reported to be found in 23 of 39 patients (59%) with surgically unrepaired BA[1].

We herein report two infants: one infant with BA who initially presented with a bone fracture before Kasai hepatic portoenterostomy, and the other at 4 wk after Kasai hepatic portoenterostomy. There are a number of factors which may be important in the etiology of bone fractures in children, including trauma, metabolic bone disease, drugs, and immobilization[3]. However, the lack of significant trauma in the majority of cases (91%) is a notable feature in children with BA[3]. Hill *et al*[3] reported 12 (19%) children with fractures before and after transplantation out of 63 undergoing liver transplanatation. Eight of 12 children with fractures in BA had no identifiable trauma. The age at the time of fracture in BA ranged from 3 to 16 mo after birth, and the affected children suffered from osteopenia (generalized reduction in bone density). The fracture site was the ribs or long bones, and multiple fractures were seen in 2 children with BA (7 and 8 mo after birth). However, Hill *et al*[3] did not describe administering vitamin D supplements. BA patients with severe cholestasis have a risk of bone fracture despite the administration of essential vitamins and minerals such as our cases. In our cases, BA was diagnosed at 6 mo after birth in case 1 and at 3 mo after birth in case 2, with suspected severe cholestasis.

Conservative management such as immobilization using plaster bandages is generally effective for fractures in BA, and there were no complications related to fractures in our cases. In the literature, internal fixation was required in one case with oxalosis for a fractured neck of the femur[1]. The early diagnosis and treatment of BA before the occurrence of bone fracture is important. The measurement of reflected light from the surface of feces by near infrared reflectance spectroscopy was introduced by Akiyama *et al*[9] for the differential diagnosis of cholestatic diseases in infants. Another method, mass screening using color picture cards depicting normal and acholic stools, was carried out at 1 and 2 mo after birth in a Japanese prefecture[10]. Eight cases of BA were detected using this mass screening method during a 3-year period, with a specificity of 99.9% and a sensitivity of 80.0%. Such screening procedures could result in improved detection of BA in infants before bone disorders occur.

In summary, clinical awareness of BA should be maintained both in terms of careful handling to prevent possible bone fracturing and also in considering fractures as a possible diagnostic factor in children with reluctance to use a limb, even in the absence of previous trauma, before Kasai hepatic portoenterostomy. Radiological awareness is also important to avoid missing unsuspected fractures on radiographs.

REFERENCES

1 **Kobayashi A**, Kawai S, Utsunomiya T, Obe Y. Bone disease in infants and children with hepatobiliary disease. *Arch Dis Child* 1974; **49**: 641-646

2 **Toki A**, Todani T, Watanabe Y, Sato Y, Ogura K, Yoshikawa M, Yamamoto S, Wang ZQ. Bone mineral analysis in patients with biliary atresia after successful Kasai procedure. *Tohoku J Exp Med* 1997; **181**: 213-216

3 **Hill SA**, Kelly DA, John PR. Bone fractures in children undergoing orthotopic liver transplantation. *Pediatr Radiol* 1995; **25** Suppl 1: S112-S117

4 **Katsura S**, Ogita K, Taguchi T, Suita S, Yoshizumi T, Soejima Y, Shimada M, Maehara Y. Effect of liver transplantation on multiple bone fractures in an infant with end-stage biliary atresia: a case report. *Pediatr Surg Int* 2005; **21**: 47-49

5 **Kasai M**, Sawaguchi S, Akiyama T, Saito J, Suruga K, Kira J, Ueta T, Okamoto E, Kimura S, Ikeda K. A proposal of new classification of biliary atresia. *J Jpn Soc Pediatr Surg* 1976; **12:** 327-331

6 **Hashizume K**, Nakajo T, Naito H, Naito T, Aso S, Aso K, Omiya T, Kamamorita K. Hemorrhagic disease of the infant accompanied with biliary atresia. *J Jpn Soc Pediatr Surg* 1980; **16:** 561-568

7 **Argao EA**, Specker BL, Heubi JE. Bone mineral content in infants and children with chronic cholestatic liver disease. *Pediatrics* 1993; **91**: 1151-1154

8 **Chongsrisawat V**, Ruttanamongkol P, Chaiwatanarat T, Chandrakamol B, Poovorawan Y. Bone density and 25-hydroxyvitamin D level in extrahepatic biliary atresia. *Pediatr Surg Int* 2001; **17**: 604-608

9 **Akiyama T**, Yamauchi Y. Use of near infrared reflectance spectroscopy in the screening for biliary atresia. *J Pediatr Surg* 1994; **29**: 645-647

10 **Maki T**, Sumasaki R, Matsui, A. Biliary Atresia: Recent Findings. Mass Screening for Biliary Atresia. *Jpn J Pediatr Surg* 1999; **31:** 242-246

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Toshiya Kamiyama

Identification of novel serum biomarkers of hepatocellular carcinoma using glycomic analysis

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Key words: hepatocellular carcinoma; glycomics; hepatectomy; biomarker.

Conflicts of interest

The authors declare no conflicts of interest.

Abstract

Background: The altered *N*-glycosylation of glycoproteins has been suggested to play an important role in the behavior of malignant cells. Using novel glycomics technology, we attempted to determine the specific and detailed *N*-glycan profile for hepatocellular carcinoma (HCC) and investigate the prognostic capabilities.

Method: From 1999 to 2011, 369 patients underwent primary curative hepatectomy in our facility and were followed up for a median of 60.7 months. As normal controls, Japanese 26 living related liver transplantation donors were selected not infected by hepatitis B and C virus. Their mean age was 40.0. Fifteen (57.7%) were male. We used a glycoblotting method to purify *N*-glycans from preoperative blood samples from this cohort (10μl serum) which were then identified and quantified using mass spectrometry (MS). Correlations between the *N*-glycan levels and the clinicopathologic characteristics and outcomes for these patients were evaluated.

Results: Our analysis of the relative areas of all the sugar peaks identified by MS, totaling 67 *N*-glycans, revealed that a proportion had higher relative areas in the HCC cases compared with the normal controls. Fourteen of these molecules had an area under the curve of greater than 0.80. Analysis of the correlation between these 14 *N*-glycans and surgical outcomes by univariate and multivariate analysis identified G2890 (*m/z* value, 2890.052) as significant recurrent factor and G3560 (*m/z* value,

3560.295) as significant prognostic factor. G2890 and G3560 were found to be strongly correlated with tumor number, size and vascular invasion.

Conclusion: Quantitative glycoblotting based on whole serum *N*-glycan profiling is an effective approach to screening for new biomarkers. The G2890 and G3560 *N*-glycans determined by tumor glycomics appear to be promising biomarkers for malignant behavior in HCCs.

Abbreviations

HCC: hepatocellular carcinoma

PS: patient Survival

DFS: disease-free survival

RF: risk factor

ICGR15: indocyanin green retention rate at 15 minutes

AFP: alpha-fetoprotein

PIVKA-II: protein induced by vitamin K absence or antagonism factor II

AFP-L3: Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

AUC: area under the curve

ROC: receiver operating characteristics

Introduction

Hepatocellular carcinoma (HCC) is a common and fatal malignancy with a worldwide occurrence (1). Liver resection has shown the highest level of control among the local treatments for HCC and is associated with a good survival rate (2, 3). However, the recurrence rates for HCC are still high even when a curative hepatectomy is performed (4). Many factors associated with the prognosis and recurrence of HCC have now been reported. Vascular invasion of the portal vein and/or hepatic vein and tumor differentiation are important factors affecting survival and recurrence in HCC cases after a hepatectomy (5, 6). However, microvascular invasion and differentiation can only be detected by pathological examination just after a hepatectomy, and cannot be diagnosed preoperatively, this cannot be identified preoperatively either. Hence, the serum biomarkers alpha-fetoprotein (AFP) and protein induced by vitamin K absence-II (PIVKA-II) are used as prognostic markers (7, 8) and also as surrogate markers for microvascular invasion and tumor differentiation(9, 10). AFP is associated with grade differentiation (11), whereas PIVKA-II is related to vascular invasion (12, 13). However, these tumor markers have limited sensitivity and are less predictive than microvascular invasion (14, 15) which is the most potent determinant of recurrence and survival in HCC patients undergoing a hepatectomy(5). Therefore, new biomarkers that are more strongly

associated with prognosis and recurrence in HCC than AFP or PIVKA-II are highly desirable.

Glycosylation is one of the most common post-translational protein modifications. Alterations in the *N*-glycosylation profiles of glycoproteins have been suggested to play important roles in the proliferation, differentiation, invasion and metastasis of malignant cells. Glycan species can be analyzed and characterized using mass spectrometry and the profiling of these molecules when they are secreted or shed from cancer cells is also performed. Hence, some glycoproteins have been suggested as biomarkers of human carcinomas such as ovarian cancer, breast cancer and HCC(16-19). Of note, changes to the N-linked glycan modification of glycoproteins occur during the tumorigenesis and progression of HCC lesions. However, the correlation between the *N*-glycan profile and tumor-associated characteristics such as the degree of malignancy and prognosis has not been previously evaluated in HCC. Recently, we developed a novel glycomics method that facilitates high-throughput and large scale glycome analysis using an automated glycan purification system, SweetBlot. This approach enables us to profile serum *N*-glycans quantitatively. Using this quantitative *N*-glycomics procedure *via* glycoblotting technology, which is both highly accurate and can be conducted on a large scale, we have previously evaluated the potential of using *N*-glycans as markers

of the prognosis and recurrence of HCC (20).

In our current study, we have evaluated preoperative blood samples from a HCC patient cohort from which we purified serum *N*-glycans using our glycoblotting method (21, 22). We performed *N*-glycan profiling using mass spectrometry to search for factors related to prognosis and recurrence by analysis of patient outcomes in 369 consecutive HCC cases that had undergone a primary curative hepatectomy at our medical facility. We sought through this screen to correlate *N*-glycan levels on glycoproteins with the clinicopathologic characteristics and the outcomes of HCC.

Methods

Patients

Between April 1999 and March 2011, 369 consecutive adult patients underwent a hepatectomy procedure for HCC at our center and this sample population was examined in the current study. Patients with extrahepatic metastases had been excluded from this cohort because the outcomes of a hepatectomy in these cases are typically very poor. The mean age of the patients in the final study group was 62.7 ± 10.6 years (range, 33-90), 301/369 (81.6%) cases were male, 176 (47.7%) were hepatitis B virus surface antigen-positive, 119 (32.2%) were hepatitis C virus antibody-positive, and 120 (32.5%) were designated as F4 based on the New