174例)によれば，約半数の43％にあたる患者が，特別な社会医療保障制度を受けていないと回答している．また，適切な医療費助成を受けている患者においても，「手続きが煩雑でわかりにくい」，「審査が厳しい」，「自治体による対応に差がある」などの不満があげられており，今後の整備が急務であることを示している．

4 おわりに

概して小児肝疾患の成人期肝移植の成績は小児期肝移植と遜色ない成績である．そうした中で本稿では，小児肝疾患の成人期肝移植が抱えるさまざまな問題点について概説した．周術期の合併症対策もさることながら，今後，transitional careの問題や医療費の公的補助，就労などに対する社会的保障制度など，患者本人や家族が安心して必要な医療，福祉を受けられるシステムがますます整備されることを願っている．

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Original Article

Pre- and postoperative nutritional assessment and health-related quality of life in recipients of living donor liver transplantation

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Aim: The nutritional state of living donor liver transplantation (LDLT) recipients is one of the most important factors affecting postoperative outcome. Although the assessment of health-related quality of life (HRQOL) is of increasing importance, few studies have examined this in conjunction with LDLT recipient nutritional state.

Methods: Ten LDLT recipients with end-stage liver disease were recruited for this study. Measurements of energy expenditure, anthropometrics and laboratory data were performed before and 1, 6 and 12–24 months after LDLT. HRQOL was measured by using the 36-item Short-Form (SF-36) before and 1, 3, 6 and 12–24 months after LDLT.

Results: The preoperative value of non-protein respiratory quotient (npRQ) was 0.796 ± 0.026 and it increased significantly after the operation. Serum non-esterified fatty acid (NEFA) levels were high in the preoperative state, but had significantly decreased 1 month after the operation. A negative correlation between npRQ and NEFA was observed throughout the study period. Cholinesterase and albumin levels improved to normal levels within 6 and 12–24 months, respectively. The recovery of the physical component summary of the SF-36 was observed after the improvement of all domains of laboratory data and energy metabolism based on the nutritional state.

Conclusion: This study demonstrated that the recovery of metabolic function, laboratory data and HRQOL in LDLT recipients are variable, and it took more than 6 months to normalize the liver protein synthetic capacity and physical HRQOL score periods. Therefore, long-term nutritional support is required in LDLT recipients.

Key words: energy metabolism, living donor liver transplantation, non-protein respiratory quotient, nutritional assessment, quality of life

INTRODUCTION

LIVER TRANSPLANTATION IS the accepted treatment for patients with end-stage liver disease (ESLD). The outcome for liver transplantation patients has improved markedly in recent years as a result of advances in immunosuppressive protocols, preservation techniques and postoperative management.[1] In Japan, a total of 4292 living donor liver transplantations (LDLT) have been performed in 2006, and 2621 of these were adult-to-adult LDLT. The overall 3- and 5-year patient survival rates were 73.8% and 70.4%, respectively.[2]

Living donor liver transplantation recipients' malnutrition was found to be associated with increased length of stays in the intensive care unit (ICU), mortality and total hospital charges.[3,4] Therefore, adequate nutritional management and therapy are required to avoid malnutrition and the associated risks. However, there have been few studies which have performed nutritional assessment of LDLT patients.

The majority of patients with ESLD have decreased respiratory quotient (RQ) and increased resting energy expenditure (REE).[5,6] Low RQ is associated with decreased glucose oxidation and increased fat oxidation[7] and is indicative of starvation, such as which can

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occur after an overnight fast due to a lack of glycogen storage. For this reason, an increase in non-protein RQ (npRQ) values can be used as a marker of recovery in chronic liver disease patients.[8] Tajika *et al.*[9] also found that the npRQ represented an independent risk factor for survival in cirrhotic patients as individuals with a lower npRQ had a worse prognosis. Hypermetabolism may contribute to the protein energy malnutrition (PEM) associated with liver disease[10] as an increased REE has been reported in cirrhotic patients.[5] However, a definitive connection cannot be made as other reports have described normal or decreased REE.[7,11] In addition, a longitudinal study reported that postoperative hypermetabolism peaked on day 10 after transplantation, and continued to the hypermetabolic state over the following 6 months.[12] By 12 months post-transplant, there was no longer a difference between the measured and predicted basal metabolic rates.[13] Despite these studies, very little information is available which describes REE and RQ changes over the long term after LDLT.

The goal of transplantation is not only to ensure patient survival, but also to return a similar state of health as was enjoyed before the disease. This requires achieving a balance between the functional efficacy of the graft and the patient's physical and psychological integrity. The assessment of the health-related quality of life (HRQOL) is increasingly used as an outcome measure when evaluating medical procedures.[14] Although numerous studies have reported significantly improved HRQOL compared with the preoperative state,[15,16] the precise timing of the improvement is often debated. In addition, most studies that have investigated HRQOL following LDLT have not included measurements of energy metabolism, which is the basis of nutritional therapy, such as RQ.

In this study, we therefore performed nutritional assessment, including energy metabolism based on nutritional state, laboratory data and HRQOL, in both the pre- and postoperative states of LDLT recipients.

METHODS

Patients

THIS STUDY WAS conducted at Tokushima University Hospital. Ten recipients and eight control subjects were recruited for the study. The study design was approved by the ethical committee of Tokushima University Hospital. Written informed consent was obtained from each patient.

Anthropometric and food intake data

Bodyweight (BW) and body mass index (BMI) were measured under fasting conditions using a TBF-102 body composition meter (Tanita, Tokyo, Japan). Before LDLT, the dry weight was calculated by deducting an estimated weight for ascites in patients with ascites. Dieticians interviewed the amount of food eaten (meals + snacks), and asked the dietary intake by 24-h recall method and calculated energy intake. Under a dietitian's advice, a recommended energy intake of 30–35 kcal/kg was adjusted depending on their activity, with a protein intake of 1.0–1.2 g/kg and fat intake of below 50 g/day. Patients with inadequate food intake received supplemental enteral nutrient. A dietitian checked BW at every measurement day by indirect calorimetry and instructed on maintaining adequate BW.

Laboratory data

Serum biochemical parameters (white blood cells [WBC], red blood cells [RBC], hemoglobin [HGB], platelets [PLT], aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin [T-bil], direct bilirubin [D-bil], γ-glutamyltransferase [GGT], total protein [TP], albumin [Alb], cholinesterase [ChE], ammonia [NH_3] and C-reactive protein [CRP]) were measured prior to and 1, 6 and 12–24 months after LDLT. Blood samples were taken to determine the concentrations of fasting blood glucose (FBG) and non-esterified fatty acids (NEFA) at the indirect calorimetry measurements.

Energy measurements

Measurements of energy expenditure were made before and 1, 6, and 12–24 months after LDLT. Indirect calorimetry measurements were carried out at 07.30 hours after overnight fasting using an AE-300S respiratory gas analyzer (Minato Medical Science, Osaka, Japan). The O_2 consumption and CO_2 production rates were calculated, and once an equilibrium steady state was achieved, these values were used to calculate the REE. The basal energy expenditure (BEE) was estimated according to the equation reported by Harris and Benedict,[17] and the ratio of REE to BEE was expressed as the %REE. Urine was collected to assay the amount of nitrogen excretion. The npRQ was calculated from measurements of the daily urinary nitrogen excretion.

HRQOL

Questionnaires were completed prior to and 1, 3, 6 and 12–24 months after LDLT. HRQOL was assessed by the Short-Form Version 2 (SF-36v2)[18,19] which consists of

eight categories, including physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH). It also includes two summary areas, with one representing a physical component summary (PCS) and the other a mental component summary (MCS). The raw scores were linearly transformed with standard scoring algorithms yielding scores that were then further adjusted using a Japanese norm-based scoring system to generate normalized scores with a mean (standard deviation) of 50 ± 10 (norm-based scores [NBS]).[20]

Statistical analysis

All data are expressed as mean ± standard error. Statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS, Chicago, IL, USA). Repeated measures ANOVA with subsequent Dunnett's test were used to assess postoperative changes from the preoperative state. Differences between recipients and control subjects were analyzed with the use of Student's *t*-test. QOL score of LDLT recipient before and after LDLT was compared with healthy control subjects at baseline using Student's *t*-test with Bonferroni correction. Pearson's correlation coefficient analysis and simple regression were used to assess the relationship between npRQ and serum NEFA levels. *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Patients

THE CHARACTERISTICS OF the LDLT patients and control subjects are listed in Table 1. LDLT was performed using the left and caudate lobe for all patients. The severity of postoperative complications was graded according to the Clavien–Dindo classification. Continuous hemodiafiltration was performed in case 7 at 13 days after LDLT, because the patient was complicated with acute renal failure (grade IV-a). Case 1 suffered from supraventricular arrhythmia and case 3 suffered from hemophagocytic syndrome (grade II). Other patients did not have severe complications after LDLT (grade I). None of the patients required reoperation for complications arising from the transplant operation. No patients suffered long-term complications associated with LDLT, such as biliary tract stricture and chronic rejection. The control group consisted of eight healthy individuals who donated part of the liver.

Table 1 Characteristics of LDLT recipients and control subjects

Case	Sex	Age (years)	Bodyweight (kg)	Body mass index (kg/m²)	MELD score	Child–Pugh		Diagnosis	Period in ICU (days)	Period in hospital (days)	Graft	
						Grade	Score				(g)	GV/SLV (%)
1	M	55	54.5	20.8	16.0	C	13	LC (HCV)/HCC	7	46	396	34.2
2	M	55	67.9	24.3	24.5	C	10	LC (HBV)/FH	7	72	515	41.3
3	F	52	52.9	23.8	12.6	C	11	LC (HCV)/HCC	12	50	510	47.8
4	M	56	61.3	20.5	14.5	C	13	LC (HBV)	7	44	450	36.8
5	M	66	50.1	17.9	14.0	B	9	LC (HCV)/HCC	11	35	420	37.8
6	F	57	52.5	22.7	18.5	C	12	LC (non-B, non-C)	7	50	460	45.6
7	F	59	63.4	27.1	17.8	C	13	LC (HCV)	25	85	385	33.9
8	F	38	57.0	22.5	13.1	C	10	LC (HBV)/HCC	8	37	390	35.2
9	F	56	83.9	28.7	16.3	C	10	LC (HCV)/HCC	6	70	520	37.5
10	F	57	55.9	21.6	27.2	C	11	LF (autoimmune)	8	73	370	32.1
Recipient ($n = 10$)	M4/F6	55.1 ± 2.2	59.9 ± 3.2	23.0 ± 1.0	17.4 ± 1.5		11.4 ± 0.5		9.8 ± 1.8	56.2 ± 5.5	441.6 ± 20.5	38.2 ± 1.8
Control ($n = 8$)	M7/F1	48.8 ± 4.8	58.6 ± 1.9	20.9 ± 0.6								

Values are expressed as mean ± standard error.
FH, fulminant hepatitis; GV/SLV, graft volume/standard liver volume; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICU, intensive care unit; LC, liver cirrhosis; LF, liver failure; MELD, model for end-stage liver disease.

Table 2 Body composition and energy intake

	Control	Before LDLT	After LDLT		
			1 month	6 months	12–24 months
Body composition					
BW (kg)	58.6 ± 1.9	59.9 ± 3.2	56.5 ± 3.6	56.0 ± 2.0	59.6 ± 3.9
BMI (kg/m^2)	20.9 ± 0.6	23.0 ± 1.0	21.7 ± 1.3	21.1 ± 0.6	22.8 ± 1.1
Energy intake					
Total (kcal/day)	1996 ± 71	1570 ± 102†	1736 ± 173	1823 ± 125	1873 ± 101
per kg of BW (kcal/kg per day)	34.3 ± 1.7	26.8 ± 4.0†	31.5 ± 1.4	33.2 ± 0.1	31.2 ± 0.1

Values are expressed as mean ± S.E.
†$P < 0.05$ vs control value (unpaired Student's *t*-test).
BMI, body mass index; BW, bodyweight; LDLT, living donor liver transplantation.

Body composition and dietary intake

Table 2 lists the measurement data for BW, BMI and dietary intake. The mean BW decreased 3.4 ± 1.4 kg in the first month after the operation. An identical trend was observed for BMI. However, there were no significant differences in BW and BMI among postoperative measurements compared with the preoperative values.

The patient preoperative dietary intake was 26.8 ± 4.0 kcal/kg per day, while the postoperative dietary intake was 31.5 ± 1.4, 33.2 ± 0.1 and 31.2 ± 0.1 kcal/kg per day at 1, 6 and 12–24 months, respectively, after LDLT. The postoperative values also did not significantly differ from those obtained before the transplant.

Laboratory data

The serum of LDLT recipients and control patients were subjected to various biochemical analyses (Table 3). The WBC count did not differ significantly at each postoperative time point compared with preoperative value. However, the RBC count and HGB levels had not returned to normal levels after 12–24 months, while the PLT numbers significantly increased in all periods after LDLT. Both T-bil and D-bil were higher in preoperative measurements compared with normal levels, but they had returned to normal levels after 6 months. Although GGT did not significantly differ at most postoperative time points compared with the preoperative value, it increased during the first 6 months after LDLT. After an initial decrease, TP returned to normal levels after 6 months. Alb was significantly higher in both the 1- and 6-month measurements after LDLT, but it returned to the lower limit of normal levels after 12–24 months. FBG did not significantly change at each postoperative point compared with the preoperative value. Although the amount of NH_3 was higher in the preoperative sample compared with normal levels, it significantly decreased in all periods after LDLT. ChE significantly increased after LDLT from its initially low level in the 6- and 12–24-month serum samples and had reached normal levels after 6 months. The preoperative levels of NEFA were higher than the normal levels and significantly decreased after the operation. In addition, simple regression analysis revealed a negative correlation between npRQ and serum NEFA concentrations for samples regardless of when they were collected ($r = -0.624$, $P < 0.001$; Fig. 1).

Nutritional metabolism

Although there was a great variability in the anthropometric and laboratory data between individuals, the preoperative mean of the npRQ value was consistently low (0.796 ± 0.026). However, it increased to 0.888 ±

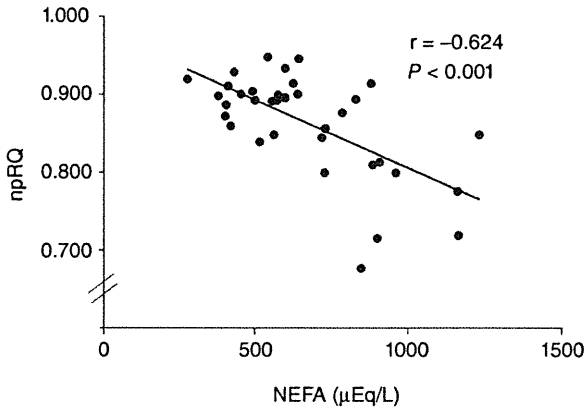

Figure 1 Relationship between npRQ and serum NEFA levels. ($P < 0.001$; Pearson's correlation coefficient analysis). NEFA, non-esterified fatty acids; npRQ, non-protein respiratory quotient.

Table 3 Laboratory data in LDLT recipients and control subjects

	Control	Before LDLT	After LDLT		
			1 month	6 months	12–24 months
WBC (/μL)	6163 ± 826	4440 ± 1217	6470 ± 1391	5478 ± 797	5544 ± 665
RBC ($\times 10^6$/μL)	4.28 ± 0.13	3.19 ± 0.18†	3.19 ± 0.15	3.89 ± 0.28*	4.10 ± 0.30*
HGB (g/dL)	12.5 ± 0.5	10.8 ± 0.5†	10.6 ± 0.5	12.0 ± 0.8	12.9 ± 0.9*
PLT ($\times 10^4$/μL)	25.6 ± 1.7	7.3 ± 1.6†	29.5 ± 4.2*	22.6 ± 2.9*	22.5 ± 1.7*
AST (IU/L)	17 ± 1	71 ± 18†	61 ± 26	30 ± 8	29 ± 7
ALT (IU/L)	17 ± 3	42 ± 12	62 ± 17	23 ± 6	16 ± 2
T-bil (mg/dL)	0.8 ± 0.1	9.5 ± 4.0	2.7 ± 1.4	0.9 ± 0.1*	1.0 ± 0.1*
D-bil (mg/dL)	0.1 ± 0.0	4.8 ± 2.8	1.4 ± 1.1	0.2 ± 0.1	0.1 ± 0.0
GGT (IU/L)	25 ± 5	30 ± 5	105 ± 18	112 ± 66	74 ± 31
TP (g/dL)	6.8 ± 0.1	6.3 ± 0.3	5.7 ± 0.1*	6.6 ± 0.1	7.1 ± 0.1*
Alb (g/dL)	3.9 ± 0.1	2.4 ± 0.1†	3.4 ± 0.1*	3.7 ± 0.2*	4.0 ± 0.1*
ChE (IU/L)	292 ± 22	77 ± 14†	132 ± 12*	299 ± 6*	309 ± 21*
NH_3 (μg/dL)	39 ± 4	91 ± 12†	43 ± 2*	41 ± 5*	35 ± 4*
CRP (mg/dL)	0.15 ± 0.08	0.64 ± 0.19†	0.74 ± 0.17	0.22 ± 0.08	0.29 ± 0.15
ICG15R (%)	5.1 0.9	45.4 ± 3.2†			
FBG (mg/dL)	94 ± 3	106 ± 9	89 ± 4	106 ± 8	113 ± 6
NEFA (μEq/L)	402 ± 42	1002 ± 112†	630 ± 66*	602 ± 17*	501 ± 45*

Values are expressed as mean ± standard error.
*$P < 0.05$ vs preoperative value (Dunnett's multiple comparison test).
†$P < 0.05$ vs control value (unpaired Student's *t*-test).
Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ChE, cholinesterase; CRP, C-reactive protein; D-bil, direct bilirubin; FBG, fasting blood glucose; GGT, γ-glutamyltransferase; HGB, hemoglobin; ICGR15, retention rate of indocyanine green in 15 min; LDLT, living donor liver transplantation; NEFA, non-esterified fatty acids; NH_3, ammonia; PLT, platelet; RBC, red blood cell; T-bil, total bilirubin; TP, total protein; WBC, white blood cell.

0.011, 0.895 ± 0.009 and 0.892 ± 0.010 at 1, 6 and 12–24 months, respectively, after the operation, which represented a significant difference when compared to the preoperative value (Fig. 2). In addition, although the npRQ was lower in the recipient group than the control group prior to LDLT, there was no significant difference between these groups after LDLT.

In this study, the %REE (86.4 ± 4.3) in the preoperative state was similar with the values determined for the control group. After LDLT, the %REE increased to 92.3 ± 2.8% during the first month, and significantly increased to 98.7 ± 3.1% and 98.3 ± 2.6% at 6 and 12–24 months, respectively, after the operation.

HRQOL

During the preoperative period, the mean HRQOL scores for all of the represented scales were below the control group and Japanese NBS. One physical (PF) and two psychological (VT and SF) components were significantly improved after 12–24 months compared with the preoperative values (Fig. 3). Although the PCS was also significantly improved after 12–24 months, this score remained lower than the control group and Japanese NBS. In contrast, the MCS did not differ at each time point compared with the control group and Japanese NBS.

DISCUSSION

AS THE LIVER plays a central role in fuel and energy metabolism, protein-energy malnutrition is common in patients with liver cirrhosis due to abnormal fuel metabolism. Energy metabolism is unbalanced in patients with a poor nutritional status, as demonstrated by increased and decreased rates of lipid and glucose oxidation, respectively. In this study, the npRQ of the patients prior to LDLT was initially low; however, it significantly increased after the operation. Thus, patients had more calories derived from fat and fewer calories derived from carbohydrates before LDLT due to decreased glycogen storage as a result of liver disorder and is consistent with a previous report.[10] Moreover, a decreased npRQ in the fasting state in cirrhotic patients would induce an elevation of serum NEFA concentra-

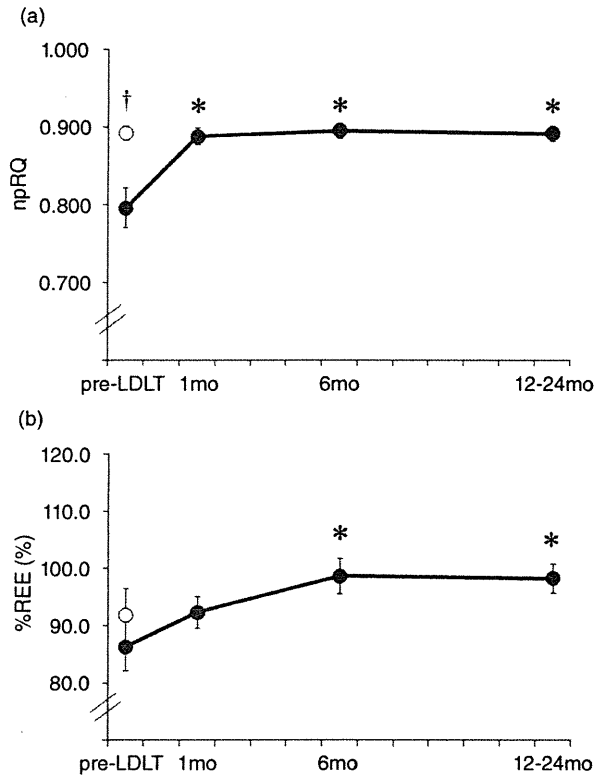

Figure 2 (a) npRQ and (b) %REE before and after LDLT. Open squares indicate control; filled squares indicate LDLT recipients. Values are expressed as mean ± standard error. $^*P < 0.05$ vs preoperative value (Dunnett's multiple comparison test). †$P < 0.05$ vs control value (unpaired Student's *t*-test). %REE, resting energy expenditure/basal energy expenditure; LDLT, living donor liver transplantation; npRQ, non-protein respiratory quotient. -○-, control: -●-, LDLT recipients.

tions by diminishing glucose oxidation and decrease glycogen stores in the liver and skeletal muscle.[5] Although the mean NEFA value was high before LDLT, it was significantly lower at all postoperative time points compared with the preoperative value. Moreover, a negative correlation was observed between npRQ and NEFA. For the diagnosis of npRQ, however, indirect calorimetry is required so that in daily practice most clinicians cannot use this approach. Taken together, these results suggest that the assessment of serum NEFA concentrations is a useful predictor of npRQ values, without the need for time-consuming indirect calorimetry measurements.

We also assessed changes in the laboratory data of blood serum associated with LDLT. The parameters of liver detoxification capacity, such as NH_3 and T-bil levels, immediately improved after transplantation. However, the length of time for the ChE and Alb levels, which indicate hepatic protein synthetic capacity, to improve to normal levels required 6 and 12–24 months, respectively. In a previous study, measuring the ChE activity showed liver function, which was useful for determining the prognosis of patients during the post-transplantation[21] and recovery period, which also agreed with past reports.[22] Although the mean npRQ was lower in the recipient group than the control group before LDLT, there was no significant difference between these groups 1 month after the transplantation. These results

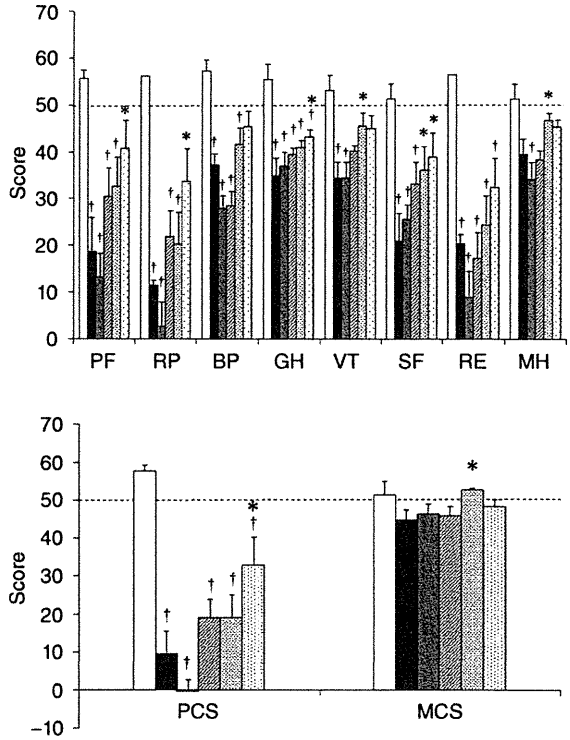

Figure 3 Normalized SF-36 scores at pre- and post-LDLT. Values are expressed as mean ± standard error. $^*P < 0.05$ vs preoperative value (Dunnett's multiple comparison test). †$P < 0.05$ vs control value (unpaired Student's *t*-test with Bonferroni correction). Fifty is the reference score of the general population. BP, bodily pain; GH, general health; LDLT, living donor liver transplantation; MCS, mental component summary; MH, mental health; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, 36-item Short-Form; VT, vitality. □, control: ■, pre-LDLT; ▩, 1 month; ▨, 3 months; ▩, 6 months; ▨, 12–24 months.

indicate that the patients' glycogen storage capacity improved to a comparable level with healthy subjects quite quickly after LDLT. Thus, although the liver detoxification and glycogen storage capacities were improved at an early postoperative period, a much longer recovery period is required to improve the hepatic protein synthetic capacity, as indicated by serum ChE and Alb levels. Therefore, these results strongly suggest that long-term nutritional management of at least 24 months is necessary in LDLT recipients.

As patients with cirrhosis have been reported to have either increased,[10] normal[7,11] or decreased[7] resting metabolic rates, this issue remains controversial. Although we have previously reported that the measured energy expenditure in Child–Pugh class A patients was typically higher than control subjects,[6] in the present study, a clear conclusion could not be reached because both hypo- and normal metabolism were observed in the liver cirrhosis patients. The variability observed in the results between these studies may have originated from differences in the degree of severity, individual variation and primary disease etiology. Thus, based on these existing variables, it would be appropriate to extend the metabolic analyses to include more cases in future studies to resolve this issue.

We also evaluated the progression of HRQOL effects before and after LDLT with the use of the Short-Form health survey, known as the SF-36, which is currently the principal tool used for reporting HRQOL changes by managed-care plans. It is also the most frequently used survey in clinical trials for a variety of interventions in a number of disorders.[19] The HRQOL of patients with severe liver cirrhosis (Child–Pugh class C) as assessed using the SF-36 were lower than those of patients with mild to moderate liver cirrhosis (Child–Pugh class A and B), as previously reported.[23–25] In this study, most patients were of Child–Pugh class C before LDLT. During the preoperative period, the patient scores for all eight scales were below the Japanese NBS, with the PCS representing the lowest value (Fig. 3). Although the HRQOL scores improved after LDLT, they tended to score below the general population in most areas even after 12–24 months, which is a trend that has been reported previously.[26] Although the PCS remained lower than the Japanese NBS, it displayed remarkable recovery within 12–24 months after LDLT. Among the factors that can affect the perception of HRQOL after LDLT, recurrent hepatitis C virus (HCV) infections and post-transplant complications were reported in previous studies to be of importance.[27,28] However, HCV did not recur in our patients after LDLT and helps explain why all HRQOL scores improved after LDLT. Our patients did not display remarkable depression on the MCS though the pre- and postoperative periods, and this may be attributed to the feeling of rebirth these patients experienced by having survived a serious illness and the greater well-being that might have accompanied this change. In addition, the recovery of PCS was observed only after all domains of laboratory data and energy metabolism based on the nutrition state were also improved. Therefore, the improvement of HRQOL encompasses a comprehensive index of progress after LDLT.

In conclusion, this study has demonstrated differences in the recovery time of nutritional metabolism function, serum biochemical data and HRQOL in LDLT patients. In particular, hepatic protein synthesis capacity and the physical score in HRQOL were shown to require a long recovery period. Therefore, it is proposed that long-term, adequate and careful nutritional care for a minimum of 2 years is required in LDLT patients. In the present study, the observed npRQ values of LDLT patients in the preoperative state were lower than the control group. As the RQ decreases after overnight fasting due to glycogen depletion in patients with liver cirrhosis, it is recommended that frequent meals and a late evening snack be consumed to correct fasting starvation in the morning.[29] The observed decrease in the npRQ in the preoperative state was thought to be due to insufficient glycogen storage in the liver. In addition, a long recovery time after LDLT is needed to improve the hepatic protein synthesis capacity. As current research indicates that branched-chain amino acid (BCAA) supplementation after hepatectomy promotes rapid improvement of protein metabolism,[30] the administration of BCAA after LDLT may be beneficial for patients' nutritional state.

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Hepatology Research

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Original Article

Cytokine expression in spleen affects progression of liver cirrhosis through liver–spleen cross-talk

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Aim: It is unclear whether the spleen affects the progression of liver cirrhosis (LC) through "liver–spleen cross-talk". Transforming growth factor-β1 (TGF-β1) is reported to be the most potent cytokine of liver fibrosis, and interleukin-6 (IL-6) is an important factor of liver regeneration. In this study, we investigated the expression of cytokines in the spleens of LC patients in order to attempt to prove the existence of liver–spleen cross-talk.

Methods: The study enrolled 22 patients who underwent splenectomy at our institute between 2004 and 2010. TGF-β1 expression in the resected spleen was measured using immunohistochemical staining. Two-color immunofluorescent staining for CD68 and TGF-β1 in the spleen was performed to detect sources of TGF-β1. IL-6 expression in the spleen was measured by reverse transcription polymerase chain reaction.

Results: TGF-β1 expression was significantly higher in the spleens of LC patients than in those of patients with normal livers ($P < 0.05$). Coexpression of CD68 and TGF-β1 was confirmed. The expression of IL-6 in the spleens of LC patients was significantly lower than that in patients with normal livers ($P < 0.05$).

Conclusion: TGF-β1 produced by macrophages and cytokines such as IL-6 could affect the progression of liver fibrosis and regeneration in patients with LC via liver–spleen cross-talk.

Key words: interleukin-6, liver cirrhosis, liver–spleen cross-talk, spleen, transforming growth factor-β1

INTRODUCTION

LIVER CIRRHOSIS (LC) is the consequence of chronic liver disease and has several possible root causes, such as alcohol consumption and viral infection. Normal liver tissue is replaced with fibrotic tissue by the pathological progression of chronic liver damage, which leads to remarkable loss of liver function. Currently, liver transplantation is considered the only curative treatment for the terminal stage of irreversible LC. However, many end-stage LC patients cannot receive a liver transplantation because of the severe shortage of cadaveric donors in some countries, such as Japan. Splenectomy has been performed as a part of Hassab's surgery for intractable esophageal or gastric varices for many years.[1–3] To date, several studies have reported that splenectomy not only decreases portal pressure and works as a radical therapy for hypersplenism but also inhibits liver fibrosis by increasing platelet count and provides some improvements in hepatic function and regeneration.[4–7] Thus, splenectomy may become an option for treatment of irreversible LC. Therefore, we focus here on the efficiency of splenectomy and cytokine expression to clarify the mechanism of LC progression via "liver–spleen cross-talk."

Transforming growth factor-β1 (TGF-β1) is the most potent cytokine of liver fibrosis; it also inhibits cell proliferation and induces apoptosis.[8–11] Kupffer cell-derived TGF-β1 activates stellate cells, thus enhancing the extracellular matrix and promoting liver fibrosis.[12] In a rat cirrhosis model, TGF-β1 expression in the spleen increased and TGF-β1 concentration in the portal vein blood decreased following splenectomy, and as a result, liver fibrosis improved.[13] In addition, the concentration of vascular endothelial growth factor in the portal vein blood was higher during liver regeneration after hepatectomy.[14] These results show that the spleen influences

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Table 1 Patients' characteristics

Factor	LC group ($n = 13$)	Normal liver group ($n = 9$)	*P*-value
Age (years)	62.1 ± 9.0	68.4 ± 15.2	0.23
Sex (M : F)	10:3	4:5	0.12
Virus background (negative : HBV : HCV)	3:1:9	9:0:0	0.0018
Platelet count ($10^4/mm^3$)	6.0 ± 2.1	21.7 ± 10.4	0.0010
Aspartate aminotransferase (IU/L)	44.4 ± 20.8	18.9 ± 3.6	0.0014
Alanine aminotransferase (IU/L)	28.3 ± 12.5	12.3 ± 3.2	0.0013
Total bilirubin (mg/dL)	1.9 ± 1.0	0.7 ± 0.3	0.0014
Prothrombin time (%)	78.7 ± 20.8	99.8 ± 35.9	0.048
Cholinesterase (U/L)	144 ± 79.9	264 ± 114.9	0.017
Indocyanine green test (%)	32.1 ± 14.5	6.9 ± 3.0	<0.001
Child–Pugh grade (A : B : C)	5:6:2	9:0:0	0.013

HBV, hepatitis B virus; HCV, hepatitis C virus; LC, liver cirrhosis.

the liver and suggest that liver–spleen cross-talk may be related to the progression of LC. However, to best of our knowledge, there have been no reports focusing on the expression of TGF-β in the spleens of cirrhotic patients.

Moreover, interleukin (IL)-6 was initially identified as a factor that promotes B-cell differentiation and production of antibodies.[15] IL-6 is an important factor of liver regeneration,[16,17] which can be triggered as IL-6 produced by Kupffer cells acts on hepatocytes.[18] However, there have been no reports investigating the expression of IL-6 in the spleens of LC patients.

In this study, we focus on the expression of cytokines in the spleens of LC patients in order to clarify the role of the spleen in the progression of LC, liver fibrosis and liver regeneration. To best of our knowledge, this is the first report to clinically investigate the possible existence of liver–spleen cross-talk in the patients with LC even though many experimental animal models have suggested that. Herein, we show our investigation of the expression of cytokines in the spleens of LC patients in order to attempt to prove the existence of liver–spleen cross-talk.

METHODS

Patients

THE STUDY ENROLLED 22 patients who underwent splenectomy at our institution between 2004 and 2011. Of these patients, 13 underwent splenectomy for esophageal or gastric varices, hypersplenism due to splenomegaly, or liver transplantation; the other nine patients had normal livers and underwent splenectomy for cancer of pancreatic body or tail, gastric cancer or benign splenic tumor. The patients' characteristics are shown in Table 1. This study was authorized in advance by the institutional review board of the University of Tokushima Graduate School, and all patients provided written informed consent.

Immunohistochemistry

Sections were prepared using the methods described previously.[19] In brief, 4-μm thick sections were cut from archival formalin-fixed, paraffin-embedded tissue blocks. The samples were deparaffinized and dehydrated using a graded series of ethanol solutions. Endogenous peroxidase activity was stopped through the administration of 0.3% hydrogen peroxidase and methanol for 20 min. After being rinsed in phosphate-buffered saline (PBS), the tissue sections were processed in a citrate buffer (0.01 M, pH 6.0) inside a heat-resistant plastic container. The sections were heated in a microwave oven for 20 min and allowed to cool at room temperature. The sections were incubated with a primary rabbit polyclonal antibody against TGF-β1 (sc-146; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. The rabbit polyclonal antibody against TGF-β1 was diluted at 1:200. After being rinsed overnight, the sections were incubated with Dako REAL Envision HRP rabbit/mouse kit (Glostrup, Denmark) for 45 min followed by three washes in PBS. Then, the peroxidase labeling was developed by incubating the section in 3,3′-diaminobenzidine-tetrachloride for 5 min. Finally, nuclear counterstaining was completed using Mayer's hematoxylin solution. All cells counts were performed using a DXM 1200F photomicroscope (Nikon, Tokyo, Japan) at a magnification of ×200. Five areas were randomly selected, and the TGF-β1 positive cells were counted for each high-power field.

Fluorescent immunostaining

Two-color immunofluorescent staining for CD68 and TGF-β1 in spleen was performed to detect derivation of TGF-β1. CD68 is a glycoprotein that binds to low-density lipoprotein, which is expressed on macrophages. Samples were prepared with the methods described previously.[19] Formalin-fixed, paraffin-embedded samples were used. Sections were serially cut at 4 μm. The sections were deparaffinized in xylene and rehydrated through a series of graded alcohols. For better antigen retrieval, the samples were put in a citrate buffer (pH 6.0) and boiled for 20 min in a microwave oven. The samples were incubated in 3% goat serum for 60 min to prevent non-specific antigen binding. The slides were incubated with primary antibodies overnight at 4°C. We used the following primary antibodies and dilutions: 1:200 dilution of a rabbit polyclonal antibody for TGF-β1 (sc-146; Santa Cruz Biotechnology) and 1:200 dilution of a mouse monoclonal antibody against CD68 (ab955; Abcam, Cambridge, UK). Primary antibody was detected with Alexa Fluor 488-conjugated antirabbit immunoglobulin (Ig)G (Invitrogen, Carlsbad, CA, USA; 1:500 dilution) and Alexa Fluor 594-conjugated antimouse IgG (Invitrogen; 1:500 dilution) for 60 min. Finally, the slides were washed in 0.1% Triton X-100 in PBS. Slides were then viewed and photographed under a confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany).

PCR quantification of mRNA expression

Total RNA was extracted using the RNeasy Mini-kit (Qiagen, Valencia, CA, USA) and reverse-transcribed with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed using the Applied Biosystems 7500 Real-Time PCR system, the TaqMan Gene Expression Assay-on-Demand and the TaqMan Universal Master Mix (Applied Biosystems). The following assays (assay identification number) were used: TGF-β1 (tHs00998133_m1), IL-6 (Hs00985639_m1), hepatocyte growth factor (HGF; Hs00300159_m1) and monocyte chemotactic protein-1 (MCP-1; Hs00234140_m1). TaqMan Human ACTB Endogenous Control (4326315E) was used as the control gene. The thermocycling conditions consisted of 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C. Amplification data were analyzed using an Applied Biosystems Prism 7500 Sequence Detection System version 1.3.1.

Statistics

All statistical analysis was performed using StatView 5.0J software (SAS Institute, Cary, NC, USA). All results are presented as mean ± standard error of the mean and were analyzed using the Mann–Whitney *U*-test and Bonferroni method. A *P*-value of 0.05 or less was considered significant.

RESULTS

Expression of TGF-β in the spleen

TRANSFORMING GROWTH FACTOR-B1 mRNA expression in the spleens of LC patients tended to be higher than that for patients with normal livers ($P = 0.13$, Fig. 1a). In a comparison of patients with normal livers and Child–Pugh classification A, B and C patients, no significant difference was found between these groups (Fig. 1b).

With regard to TGF-β1 protein expression, there were significantly more TGF-β1 positive cells in the spleens of LC patients than in the spleens of the normal liver

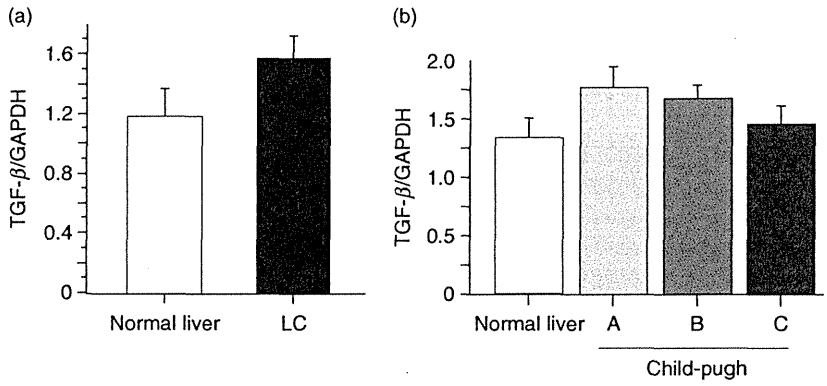

Figure 1 TGF-β1 mRNA expression in the spleen. (a) TGF-β1 mRNA expression in the spleens of liver cirrhosis patients ($n = 13$) tended to be higher than that of patients with normal livers ($n = 9$, $P = 0.13$). (b) There were no significant differences with respect to Child–Pugh classification (A : B : C = 5:6:2). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LC, liver cirrhosis; TGF-β1, transforming growth factor-β1.

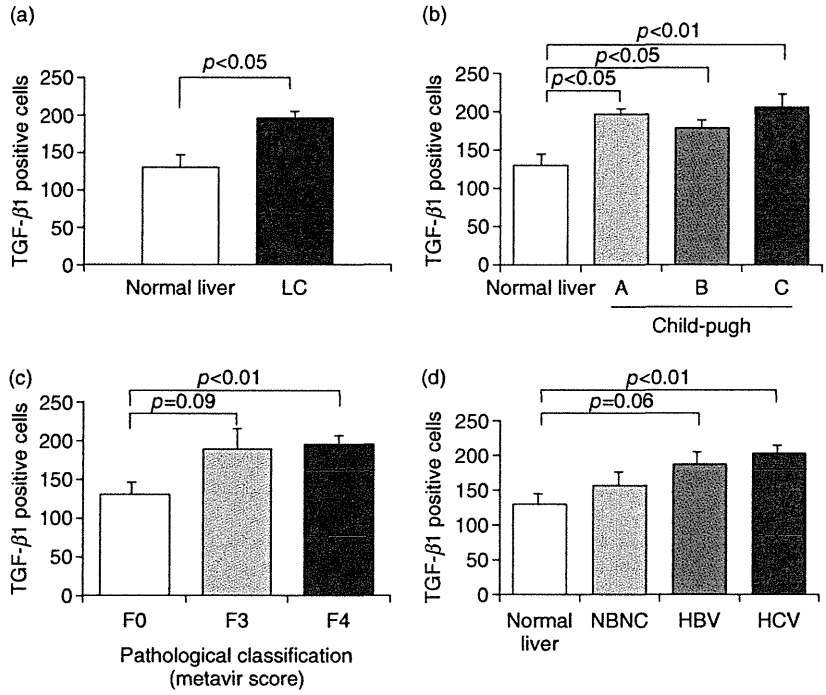

Figure 2 Immunohistochemistry showing TGF-β1 positive cells in the spleen. (a) Number of TGF-β1 positive cells in the spleens of liver cirrhosis patients ($n = 13$) was significantly higher than that in the spleens of patients with normal livers ($n = 9$, $P < 0.05$). (b) Number of TGF-β1 positive cells in the spleens of Child–Pugh classification A, B and C patients (A : B : C = 5:6:2) was significantly higher than that in the spleens of patients with normal livers ($P < 0.05$). (c) When the results were analyzed according to METAVIR score, the number of TGF-β1 positive cells in the spleens of the F4 patients was significantly higher than that of the F0 group ($P < 0.01$). (d) Number of TGF-β1 positive cells in the spleens of patients who tested positive for hepatitis C virus was significantly higher than that in the spleens of patients with normal livers (negative : HBV : HCV = 3:1:9, $P < 0.01$). HBV, hepatitis B virus; HVC, hepatitis C virus; LC, liver cirrhosis; NBNC, non-B, non-C hepatitis; TGF-β1, transforming growth factor-β1.

patients ($P < 0.05$, Fig. 2a). The numbers of TGF-β1 positive cells in the spleens of Child–Pugh classification A, B, and C patients were significantly higher than those of patients with normal livers ($P < 0.05$, Fig. 2b). On the basis of pathological classification of liver fibrosis, patients were placed in groups F0, F3 and F4 (METAVIR score). The number of TGF-β1 positive cells in the spleens of F4 patients was significantly higher than that in the spleens of F0 patients ($P < 0.01$, Fig. 2c). Furthermore, the numbers of TGF-β1 positive cells in the spleens of patients who tested positive for hepatitis C virus were significantly higher than those of the patients with normal livers ($P < 0.01$, Fig. 2d).

Fluorescent immunostaining

Coexpression of CD68 (green) and TGF-β1 (red) in the spleen was investigated using a double-colored immunofluorescent staining procedure (Fig. 3). CD68 is a glycoprotein that binds to low-density lipoprotein, which is expressed on macrophages.

Expression of cytokine mRNA in the spleen

In the spleen, the expression of IL-6 mRNA in the LC group was significantly lower than that in the normal liver group ($P < 0.05$, Fig. 4a). The expression of HGF mRNA in the LC group was significantly higher than that of the normal liver group ($P < 0.05$, Fig. 5a). MCP-1 mRNA expression in the LC group tended to be lower than that in the normal liver group ($P = 0.10$, Fig. 6a).

DISCUSSION

SEVERAL STUDIES HAVE reported that the spleen inhibited liver regeneration in animal models of LC, and it has also been reported that the cytokines produced by splenic tissue have an inhibitory affect on liver

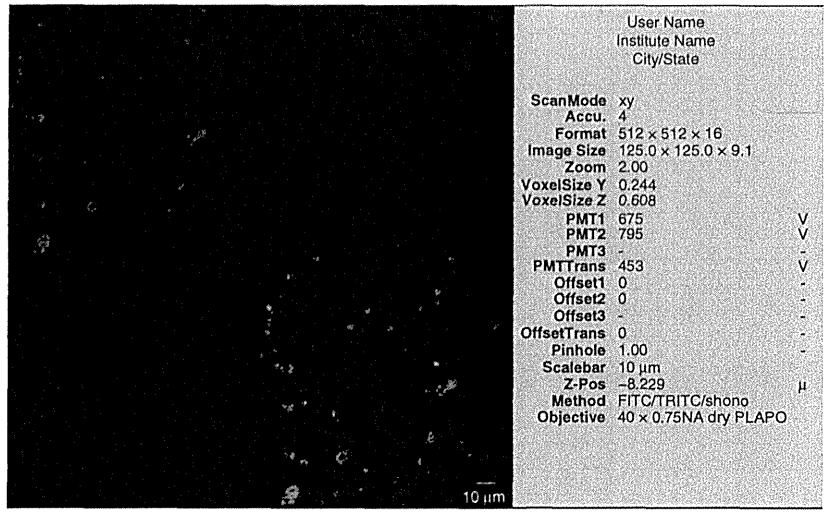

Figure 3 Two-color immunofluorescent staining for CD68 and TGF-β1 in the spleens of liver cirrhosis patients. Coexpression of CD68 (green) and TGF-β1 (red) was observed in the spleen (original magnification ×400). TGF-β1, transforming growth factor-β1.

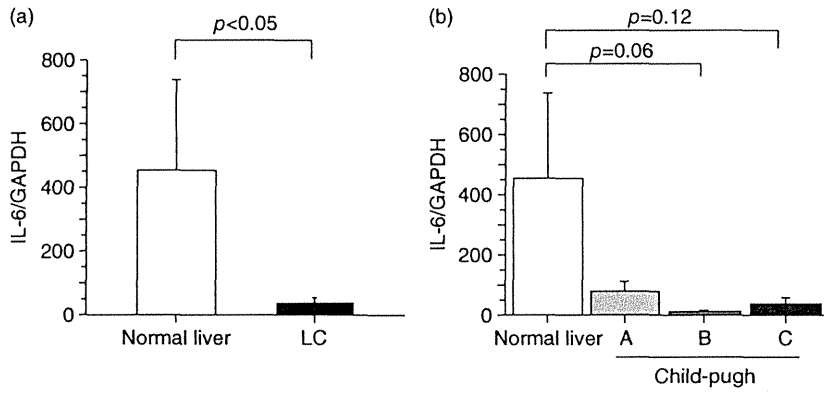

Figure 4 IL-6 mRNA expression in the spleen. (a) IL-6 mRNA expression in the LC group ($n = 13$) was significantly lower than that in the normal liver group ($n = 9$, $P < 0.05$). (b) There were no significant differences between the Child–Pugh classification groups (A : B : C = 5:6:2). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; LC, liver cirrhosis.

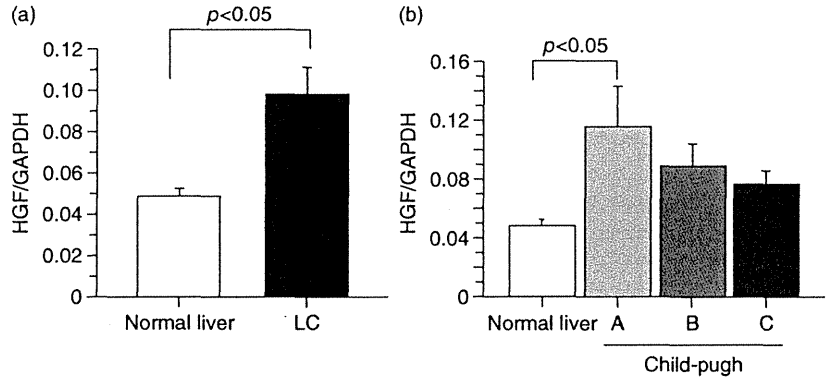

Figure 5 HGF mRNA expression in the spleen. (a) The expression of HGF mRNA in the liver cirrhosis group ($n = 13$) was significantly higher than that of the normal group ($n = 9$, $P < 0.05$). (b) With regard to Child–Pugh classification, the expression of IL-6 mRNA in the spleens of Child–Pugh classification A patients was significantly higher than that in patients with normal livers (A : B : C = 5:6:2, normal liver: $n = 9$, $P < 0.05$). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HGF, hepatocyte growth factor; LC, liver cirrhosis.

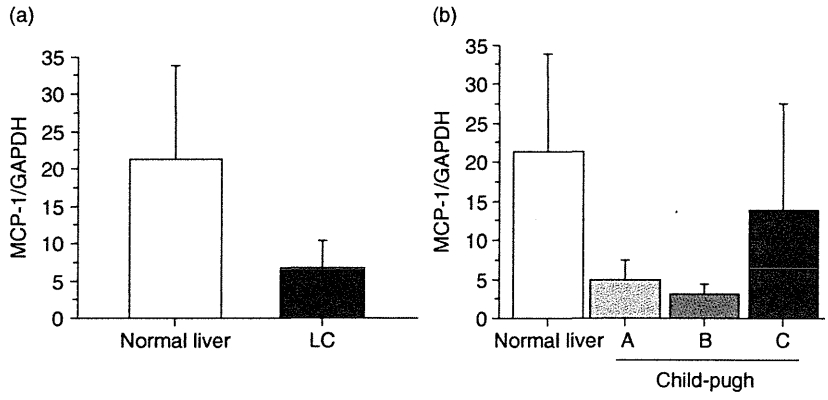

Figure 6 MCP-1 mRNA expression in the spleen. (a) There was no significant difference between the LC group ($n = 13$) and the normal group ($n = 9$). (b) There were no significant differences between the Child–Pugh classification groups. (A : B : C = 5:6:2, normal liver: $n = 9$). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LC, liver cirrhosis; MCP-1, monocyte chemotactic protein-1.

regeneration through the portal vein.[14,20] In particular, TGF-β1 expression in the spleen and plasma concentration of TGF-β1 in the portal vein increased in a rat model of LC and hepatectomy.[13,21] Splenectomy has been proven to decrease the plasma concentration of TGF-β1 in the portal vein and improve liver regeneration after hepatectomy and liver fibrosis from LC following the administration of thioacetamide in rodent models.[13,21] However, no reports have examined cytokine changes in the spleens of patients with LC.

In this study, we investigated the expression of cytokines in spleens of patients with LC. TGF-β1 expression was more elevated in the patients with LC than in those without LC. TGF-β1 mRNA expression was also higher in the patients with LC than in those without LC. TGF-β1 is the most potent cytokine for liver fibrosis and inhibits liver regeneration.[8–11] The spleen may also play an inhibitory role in patients with LC, as has been shown in rodent models. However, it will be necessary to investigate the plasma concentration of TGF-β1 in the portal vein to complete the investigation of our hypothesis. Further investigations are already planned to elucidate the importance of TGF-β1 expression in the spleen and portal vein plasma. Moreover, the small number of patients may limit the solid verification; thus, increasing their number is also required.

The derivation of TGF-β1 during liver regeneration has been controversial. Several studies have reported that TGF-β1 is secreted from hepatic mesenchymal cells in a paracrine manner and from the hepatocytes themselves in an autocrine manner.[22–24] There has been some clear evidence that the source of TGF-β1 is not only the liver but also the spleen.[13,21] We investigated the derivation cells of TGF-β1 in the spleens of patients with LC. We focused on the splenic macrophages because they produce several kinds of biologically active mediators, such as tumor necrosis factor-α (TNF-α) and IL-1β, at various states of liver damage.[25,26] Two-color immunofluorescent staining revealed the expression of CD68, the marker of macrophages, and TGF-β1. Thus, macrophages in the spleen produce TGF-β1 and could play a role in exacerbating LC by inhibiting the regeneration of hepatocytes and promoting the liver fibrosis in the damaged liver.

We also examined the expression of other cytokines in the spleens of patients with LC. The expression of IL-6 mRNA, which acts on hepatocytes as a trigger of liver regeneration, was significantly lower in the LC patients than in the patients with normal livers. Therefore, the spleen could play an inhibitory role in liver regeneration by not only producing TGF-β1 but also decreasing IL-6, which is the promoter of liver regeneration. However, expression of HGF mRNA, which is also a promoter of liver regeneration, in the spleen tended to be higher in the patients with LC than in those with healthy livers. In a rat model of LC, it has been reported that p.o. administration of thioacetamide leads to an increase of TGF-β1 concentration in serum in a time-dependent manner, and HGF concentration in serum is also augmented via feedback of LC.[27] Therefore, it is natural that similar feedback-increased HGF mRNA level could be observed in the spleen of the human patients with LC.

In LC patients, several cytokine concentrations in peripheral serum are increased, including concentrations of TNF-α, IL-8 and MCP-1.[28] This may suggest that there is liver–spleen cross-talk, in which some kind of unknown humoral factors act on the spleen and may induce cytokine expression by the spleen, which is related to the progression of LC. However, increasing the number and further study is necessary to elucidate such humoral factors that affect cytokine expression and role of the spleen in LC patients.

In conclusion, expression of TGF-β1 produced by macrophages and cytokines such as IL-6 could affect the progression of liver fibrosis and regeneration as liver–spleen cross-talk in patients with LC.

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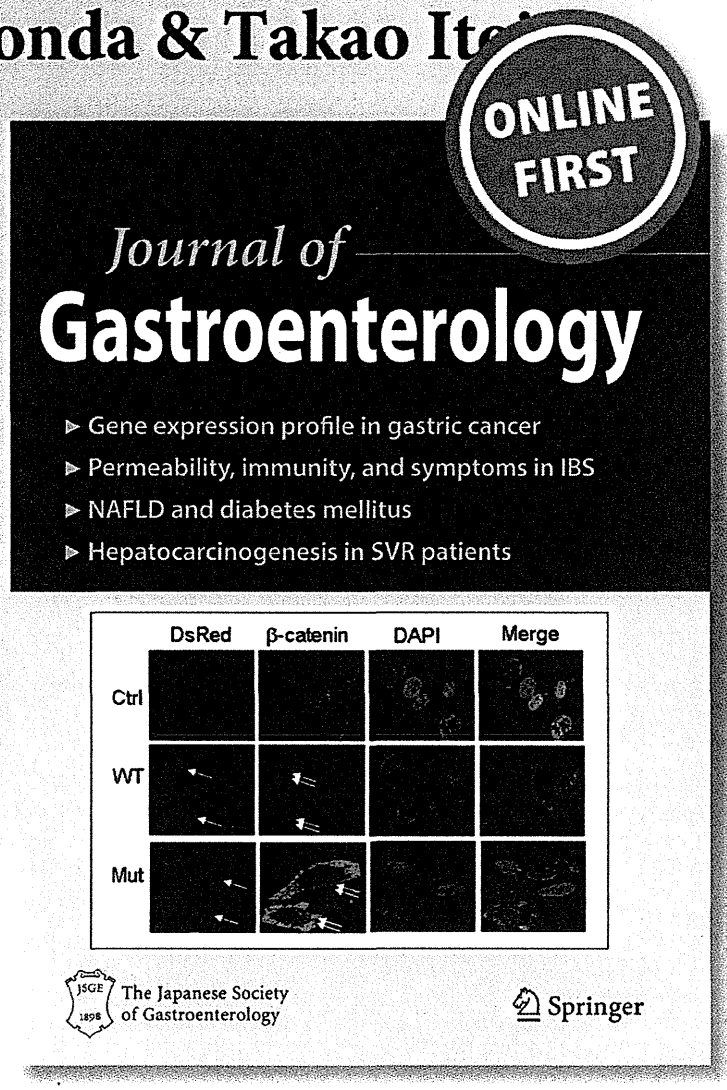

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REVIEW

Pancreaticobiliary maljunction and biliary cancer

Terumi Kamisawa · Sawako Kuruma · Taku Tabata · Kazuro Chiba · Susumu Iwasaki · Satomi Koizumi · Masanao Kurata · Goro Honda · Takao Itoi

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Abstract Pancreaticobiliary maljunction (PBM) is a congenital malformation in which the pancreatic and bile ducts join anatomically outside the duodenal wall. Japanese clinical practice guidelines on how to deal with PBM were made in 2012, representing a world first. According to the 2013 revision to the diagnostic criteria for PBM, in addition to direct cholangiography, diagnosis can be made by magnetic resonance cholangiopancreatography (MRCP), 3-dimensional drip infusion cholangiography computed tomography, endoscopic ultrasonography (US), or multiplanar reconstruction images by multidetector row computed tomography. In PBM, the common channel is so long that sphincter action does not affect the pancreaticobiliary junction, and pancreatic juice frequently refluxes into the biliary tract. Persistence of refluxed pancreatic juice injures epithelium of the biliary tract and promotes cancer development, resulting in higher rates of carcinogenesis in the biliary tract. In a nationwide survey, biliary cancer was detected in 21.6 % of adult patients with congenital biliary dilatation (bile duct cancer, 32.1 % vs. gallbladder cancer, 62.3 %) and in 42.4 % of PBM patients without biliary dilatation (bile duct cancer, 7.3 % vs. gallbladder cancer, 88.1 %). Pathophysiological conditions due to pancreatobiliary reflux occur in patients with high confluence of pancreaticobiliary ducts, a common channel ≥6 mm long, and occlusion of communication during contraction of the sphincter. Once the diagnosis of PBM is established, immediate prophylactic surgery is recommended. However, the surgical strategy for PBM without biliary dilatation remains controversial. To detect PBM without biliary dilatation early, MRCP is recommended for patients showing gallbladder wall thickening on screening US under suspicion of PBM.

Keywords Pancreaticobiliary maljunction · Congenital biliary dilatation · Gallbladder cancer · Bile duct cancer

Introduction

Pancreaticobiliary maljunction (PBM) is a congenital malformation in which the pancreatic and bile ducts join anatomically outside the duodenal wall. The sphincter of Oddi is normally located at the distal end of the pancreatic and bile ducts and regulates the outflow of bile and pancreatic juice. In PBM, the common channel is so long that action of the sphincter of Oddi does not directly affect the pancreaticobiliary junction. As a result, reciprocal reflux of pancreatic juices and bile occurs. As the fluid pressure in the pancreatic duct usually exceeds that in the bile duct, reflux of pancreatic juice into the biliary tract frequently occurs in PBM. Persistence of refluxed pancreatic juice injures the epithelium of the biliary tract and promotes cancer development, resulting in higher rates of

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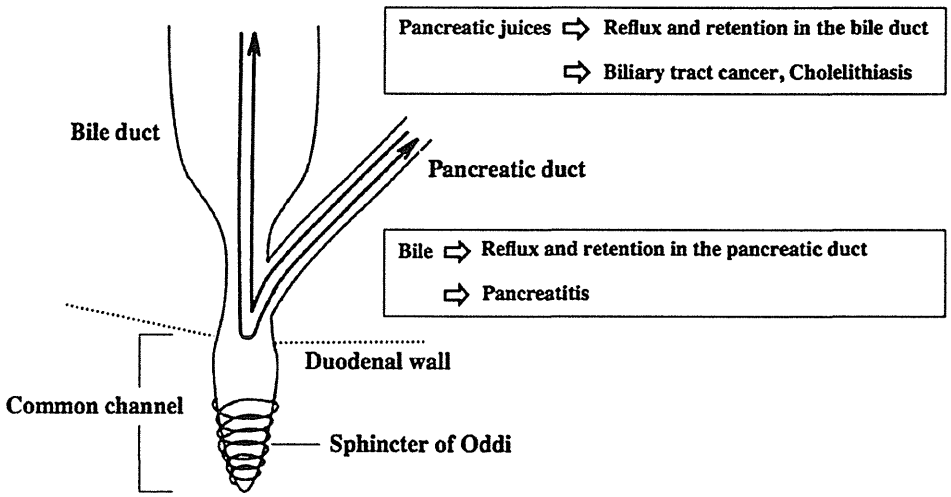

Fig. 1 The pathophysiology of pancreaticobiliary maljunction [3]

carcinogenesis in the biliary tract of PBM (Fig. 1). PBM can be divided into PBM with biliary dilatation (congenital biliary dilatation) and PBM without biliary dilatation [1, 2].

Japanese clinical practice guidelines on how to deal with PBM were created in 2012, as the first in the world [3]. Diagnostic criteria for PBM were revised in 2013, taking recent advances in diagnostic imaging techniques into consideration [4]. Based on the guidelines and new diagnostic criteria, we describe herein recent topics and problems in the management of PBM, with a focus on biliary cancer.

Diagnostic criteria for PBM 2013

Diagnostic criteria of PBM were proposed in 1987 [5], and were slightly revised in 1990 and published in English in 1994 [6]. In 2013, these criteria underwent thorough revision, 23 years to the day since the previous version (Table 1) [4]. Although no significant changes have been made to the definition of PBM, diagnostic modalities have undergone substantial advances in recent years. As no radiological modalities were initially available that could show the status of the pancreaticobiliary junction outside the duodenal wall, PBM was diagnosed when a lack of effect of the sphincter of Oddi on the pancreaticobiliary junction was verified on direct cholangiography such as with endoscopic retrograde cholangiopancreatography (ERCP).

Magnetic resonance cholangiopancreatography (MRCP) has now become popular as a noninvasive method for obtaining high-quality images of the pancreaticobiliary tree, and it is replacing diagnostic ERCP for many pancreatobiliary diseases. Many PBM cases can be diagnosed from MRCP based on findings of an anomalous union between the common bile duct and pancreatic duct in addition to a long common channel [7–10]. MRCP is thus useful for diagnosing children and screening for PBM [7]. However, accurate diagnosis of PBM is difficult in cases with a relatively short common channel (Fig. 2a, b) [11]. In cases with a common channel $\leq$9 mm on MRCP, direct cholangiography is needed to confirm PBM [12]. PBM can be diagnosed if junction outside the wall can be depicted by high-resolution images with multiplanar reconstruction (MPR) provided by multidetector row computed tomography (MD-CT), and endoscopic ultrasonography (EUS) [3, 13, 14].

Amylase levels in bile are markedly elevated (>10,000 IU/l) in most cases of PBM, but are not elevated at all in some cases [15, 16]. Furthermore, elevation of pancreatic enzyme levels in bile and hyperplastic changes to the gallbladder mucosa are sometimes observed in some cases with a relatively long common channel in which the effect of the sphincter reaches the pancreaticobiliary junction (high confluence of pancreaticobiliary ducts) [17–19].

Since the maximum diameter of the common bile duct correlates positively with age, standard values for the maximum diameter of the common bile duct in each age group appear appropriate for accurate evaluation of the presence of bile duct dilatation [20–22].

Biliary cancer associated with PBM

Incidence and characteristics

Biliary cancers are frequently observed in adult patients with PBM [23–25]. According to a nationwide survey in

Table 1 Diagnostic criteria for pancreaticobiliary maljunction 2013[4]

I. Definition

Pancreaticobiliary maljunction is a congenital malformation in which the pancreatic and bile ducts join anatomically outside the duodenal wall.

II. Pathophysiology

In pancreaticobiliary maljunction, the duodenal papillary sphincter (sphincter of Oddi) fails to exert any influence on the pancreaticobiliary junction due to the abnormally long common channel. Therefore, reciprocal reflux between pancreatic juice and bile occurs, resulting in various pathologic conditions, such as inhibiting the excretion of bile and pancreatic juice, and biliary cancer, in the biliary tract and pancreas.

III. Diagnostic criteria

Pancreaticobiliary maljunction is diagnosed by either imaging test or anatomical examination.

Imaging diagnosis

a) An abnormally long common channel and/or an abnormal union between the pancreatic and bile ducts must be evident on direct cholangiography, such as endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transpehatic cholangiography (PTC), or intraoperative cholangiography; magnetic resonance cholangiopancreatography (MRCP); or three-dimensional drip infusion cholangiography computed tomography (3D-DIC-CT). However, in cases with a relatively short common channel, it is necessary to confirm that the effect of the papillary sphincter does not extend to the junction by direct cholangiography.

b) Pancreaticobiliary maljunction can be diagnosed if the pancreaticobiliary junction outside the wall can be depicted by endoscopic ultrasonography (EUS) or multi-planar reconstruction (MPR) images provided by multi-detector row computed tomography (MD-CT).

Anatomical diagnosis

It should be confirmed by surgery or autopsy that the pancreaticobiliary junction lies outside the duodenal wall, or pancreatic and bile ducts unite abnormally.

IV. Supplementary diagnosis

The following findings strongly suggest the existence of pancreaticobiliary maljunction.

Elevated amylase levels in bile

Pancreatic enzymes, especially amylase, in the bile within the bile duct and gallbladder obtained immediately after laparotomy, endoscopically or percutaneously are generally at extremely high levels. However, levels close to or below the normal serum value are occasionally observed in patients with pancreaticobiliary maljunction.

Clinical features similar to pancreaticobiliary maljunction, including elevation of pancreatic enzymes in bile, are observed in some cases with a relatively long common channel, showing the effect of the sphincter on the pancreaticobiliary junction.

Extrahepatic bile duct dilatation

Pancreaticobiliary maljunction includes one type that is associated with bile duct dilatation (congenital biliary dilatation), and another that is not (pancreaticobiliary dilatation without biliary dilatation). When cystic, fusiform, or cylindrical dilatation is detected in the extrahepatic bile duct, careful investigations are needed to determine whether pancreaticobiliary maljunction is present.

Standard values for the maximum diameter of the common bile duct at each age are useful for diagnosing pancreaticobiliary maljunction with or without biliary dilatation.

Japan ($n = 2561$) [2], biliary cancer was detected in 21.6 % of adult patients with congenital biliary dilatation and in 42.4 % of PBM patients without biliary dilatation. In patients with biliary cancers in association with PBM, the location ratio of cancers in the bile duct and gallbladder were 32.1 % and 62.3 % in congenital biliary dilatation, and 7.3 % and 88.1 % in PBM patients without biliary dilatation, respectively. The mean age at which PBM patients developed biliary cancer was 60.1 years for gallbladder cancer and 52.0 years for bile duct cancer among patients with congenital biliary dilatation, and 58.6 years for gallbladder cancer in PBM patients without biliary dilatation. Such patients develop biliary cancers 15–20 years earlier than patients without PBM [26].

In PBM patients, biliary cancers frequently develop as simultaneous and/or metachronous double cancers. Of 37 patients with simultaneous double or multiple biliary cancers, 19 patients (51 %) suffered from concurrent PBM [3, 27–31].

The ratio of gallstone detection in PBM patients who developed gallbladder cancer was lower than that in the biliary cancer population without PBM [2, 3, 32]. In our series, the ratios were 10 % and 62 %, respectively [1].

Mechanism of biliary carcinogenesis

The mechanisms of carcinogenesis in PBM appear to be related to the persistence of refluxed pancreatic juice into the biliary tract. Refluxed proteolytic pancreatic enzymes and phospholipase A2 are activated in the biliary tract and strongly cytotoxic substances such as lysolecithin are produced. The resulting chronic inflammation provokes repeated cycles of damage and healing in the biliary mucosal epithelia. These alterations in the mucosal epithelia, in conjunction with DNA mutations, finally promote cancer development and progression (Fig. 3) [1, 3, 33, 34]. The sequence of hyperplasia-dysplasia-carcinoma, regarded as the prevailing mechanism underlying the development of biliary tract cancer in PBM, is thought to differ from both the adenoma-carcinoma sequence and de novo carcinogenesis associated with biliary tract cancer in the population without PBM [35–37].

In our series, the gallbladder mucosa was significantly higher in PBM than in controls. The incidence of epithelial hyperplasia of the gallbladder and the Ki-67 labeling index of the gallbladder epithelium were significantly higher in PBM than in controls. K-ras mutations in the noncancerous epithelium of the gallbladder were detected in 36 % of PBM patients [1, 19]. Considering that increased cell proliferation is linked to the development of cancer by means of tumor promotion and an increased rate of random mutations, the gallbladder mucosa of PBM patients can be considered to represent a premalignant region.