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Acyl/free carnitine ratio is a risk factor for hepatic steatosis after pancreatoduodenectomy and total pancreatectomy

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ABSTRACT

Objectives: Hepatic steatosis, one of the most frequent long-term complications of pancreatectomy, influences not only hepatic function but also survival rate. However, its risk factors and pathogenesis have not been established. The purpose of this study was to clarify the risk factors for hepatic steatosis after pancreatectomy.

Methods: In this retrospective study of 21 patients who had undergone pancreatectomy (19 cases of pancreatoduodenectomy and 2 cases of total pancreatectomy), serum carnitine concentrations, fractions of carnitine, and hepatic attenuation on computed tomography images were analyzed with the aim of identifying risk factors for hepatic steatosis.

Results: Thirteen (61.9%) of the 21 patients were diagnosed as having hypocarnitinemia after pancreatectomy. Average hepatic attenuation was as low as 42.2HU (±21.3 SD). A high ratio of acyl/free carnitine was associated with less pronounced hepatic attenuation according to both univariate ($P < 0.001$) and multivariate ($P = 0.020$) regression analyses.

Conclusions: The serum carnitine concentrations were low after pancreatectomy in some patients. The statistical analyses suggest that a high ratio of acyl/free carnitine is an independent risk factor for hepatic steatosis after pancreatectomy.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD), caused by hepatic steatosis as first hit and various factors as second hits [1], is a common disease with prevalence of 10–24% of various countries [2]. Risk factors for NAFLD are obesity, type 2 diabetes mellitus and hyperlipidemia [3]. The prevalence is 57.5%–74% in obese persons [4–6].

Besides obesity, pancreatectomy is another risk factor for hepatic steatosis with the prevalence of 23% [7] to 40% [8]. Persistent steatosis causes nonalcoholic steatohepatitis (NASH) [9], and liver cirrhosis or liver failure in severe cases [10]. The recurrence-free survival rate of patients with hepatic steatosis was poorer than that of patients without steatosis [11]. The characteristics of patients with hepatic steatosis after pancreatectomy are contrary to that of general obese-type NAFLD patients. Reported risk factors for hepatic steatosis after pancreatectomy are related to malnutrition [8,9,11], not with obesity. The detailed mechanisms for development of post-operative hepatic steatosis have not been revealed in details.

Thus, the general nutritional status of patients with post-operative steatosis is just opposite to that of the majority of patients with NAFLD; however, lipid metabolism is attenuated in these patients [12,13] as well as patients with general type NAFLD. Romano M et al. reported about the efficacy of L-carnitine treatment to reduce hepatic steatosis in patients with chronic hepatitis C, whose steatosis is different from that of both patients with obesity and patients after pancreatectomy [14]. We have recently reported that L-carnitine supplementation also improved hepatic

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steatosis after pancreatectomy [15]. However, the relationships between carnitine and hepatic steatosis is still unknown.

The aim of this study was to clarify the risk factors for post-pancreatectomy hepatic steatosis with particular emphasis on the status of carnitine metabolism.

2. Materials and methods

2.1. Subjects and relevant postoperative characteristics

A total of 21 pancreatic cancer patients who underwent pancreatectomy (19 cases of pancreatoduodenectomy and 2 cases of total pancreatectomy) at our institution from October 2011 through January 2014 were analyzed about risk factors for hepatic steatosis using blood carnitine-concentration and plane computed tomography (CT). Hepatic attenuation on CT images was determined by an individual average of hepatic intensities on each CT-slice. Hepatic steatosis was defined as an absolute CT attenuation of less than 40HU [8,9]. We performed uni- and multi-variate analysis using continuous variables of CT attenuation for risk analysis. Patients underwent CT examination 262 (±173 SD) days after surgery. Blood examination was performed 14 (±35 SD) days before CT. PNI (prognostic nutritional index) was calculated according to Onodera's method (PNI=(10 × serum albumin concentration)+(0.005 × total lymphocyte count) [16]. Hypocarnitinemia was diagnosed when any of the three carnitine fractions was below the normal range.

2.2. Statistical analysis

Summary statistics of the continuous variables were expressed as a mean with standard deviation (SD). For univariate analysis, linear regression model with the hepatic attenuation as a dependent variable. The binary variable was included in the model as a dummy variable in each model; the male and female, for example, were coded as 0 and 1, respectively. For multivariate analysis, the multiple linear regression model was selected to minimize Bayesean information criteria. A P value of less than 0.05 was considered statistically significant. All statistical analyses were performed using JMP 11.0.0 (SAS, Cary, NC, USA).

3. Results

3.1. Patient characteristics

As shown in Table 1, the operative procedures comprised two cases of total pancreatectomy and 19 cases of pancreatoduodenectomy. Nutritional factors including serum concentration of hemoglobin, albumin and lymphocyte count were at low level as well as PNI. The means for each of the three carnitine fractions were within the normal range. However, total, free, and acyl carnitine concentrations were below the normal range in 11, 12, and 7 patients, respectively. Twelve (61.9%) of the 21 patients were diagnosed as having hypocarnitinemia (Fig. 1). Seven (33.3%) of the 21 patients were diagnosed as hepatic steatosis. Tumor markers, CEA and CA19-9, were at high level. Average hepatic attenuation in CT images was 42.2 HU (±21.3 SD).

3.2. Risk factors for post-pancreatectomy hepatic steatosis

Diarrhea (P = 0.001*) and increase in the ratio of acyl/free-carnitine (P < 0.001*) were statistically significantly associated with hepatic steatosis after pancreatectomy by univariate analysis (Fig. 2, Table 2). The selected multivariate model with these two possible risk factors showed that only a high acyl/free carnitine

Table 1
Patient characteristics.

Gender (M/F)	13/8	
Operative procedure (TP/PD)	2/19	
	Yes/No	
Obstructive pancreatitis	14/7	
Supplementation of pancreatic enzyme	17/4	
Diarrhea	4/17	
Insulin Treatment	5/16	
	Mean	SD
The day after pancreatectomy (day)	211	197.4
Age (year)	70.8	9.6
BMI	20.4	3.0
Hb (g/dl)	11.2	1.3
Lymphocyte (/ml)	1443	647.4
Albumin (g/dl)	3.4	0.7
CRP (mg/dl)	1.5	1.9
Total cholesterol (mg/dl)	146.9	37.7
Total carnitine (mmol/l)	46.8	12.3
Free carnitine (mmol/l)	37.3	9.5
Acyl carnitine (mmol/l)	9.7	6.2
Acyl carnitine/Free carnitine	0.3	0.2
CEA (ng/ml)	12.8	30.7
CA19-9 (U/ml)	1168	2888.4
PNI	40.8	9.1
Hepatic Attenuation on CT	42.2	21.3

TP; total pancreatectomy, PD; Pancreatoduodenectomy.
BMI; Body Mass Index, Hb; hemoglobine, CRP; C reactive protein.
CEA; Carcinoembryonic antigen, PNI; prognostic nutritional index.

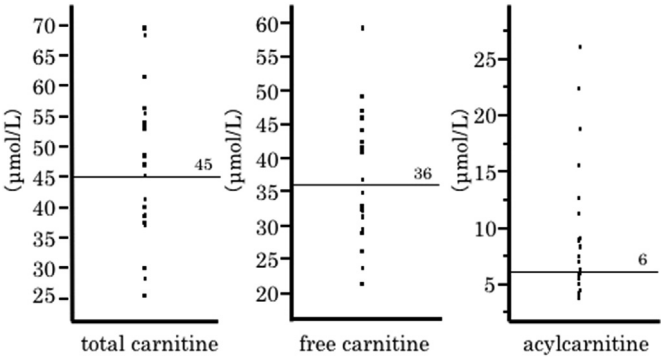

Fig. 1. Serum concentrations of carnitine fractions after pancreatectomy. The bars indicate the lower limits of the normal range for each carnitine fraction.

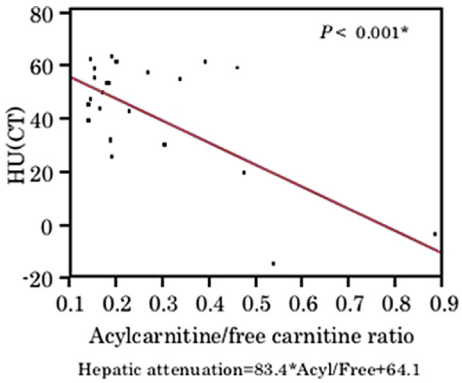

Fig. 2. Factors affecting hepatic attenuation on CT. Hepatic attenuation on CT was compared between two groups, diarrhea group and no-diarrhea group, by means of least squares method. (a). Regression plot and regression line between hepatic attenuation on CT and acyl/free carnitine ratio was made by means of least squares method (b). P value indicated results of univariate analysis. (*) indicated significant difference.

Table 2
Univariate and multivariate analysis of risk factors affecting post-operative hepatic steatosis.

	P value	
	Univariate	Multivariate
Gender	0.057	
Operative procedure	0.696	
Obstructive pancreatitis	0.506	
Supplementation of pancreatic enzyme	0.657	
Diarrhea	0.001*	0.069
Insulin treatment	0.183	
Age	0.513	
BMI	0.667	
Hb	0.905	
Lymphocyte	0.156	
Albumin	0.282	
CRP	0.886	
Total cholesterol	0.328	
Total carnitine	0.283	
Acyl/Free	<0.001*	0.020*
CEA	0.575	
CA19-9	0.320	
PNI	0.176	

ratio was statistically significantly associated with hepatic steatosis after pancreatectomy ($P = 0.020$*; Table 2).

4. Discussion

In this study, more than half the subjects had hypocarnitinemia after pancreatectomy. We also identified by univariate and multivariate analysis that a high acyl/free carnitine ratio was a risk factor for hepatic steatosis after pancreatectomy.

Univariate analysis suggested that diarrhea is a possible risk factor for hepatic steatosis. Diarrhea is a pivotal symptom that influences nutritional status after pancreatectomy; risk factors for hepatic steatosis associated with nutritional status include BMI (body mass index) [11], remnant pancreatic volume, pancreatic resection line [8,10] and diarrhea [9]. In the present study, diarrhea was associated with acyl/carnitine ratio. This may explain why the association of hepatic steatosis with diarrhea lost its statistical significance when the multiple regression model adjusted diarrhea and acyl/carnitine ratio with each other. The result of the multiple regression analysis, therefore, suggests that acyl/carnitine ratio is independently associated with hepatic steatosis after pancreatectomy. .

This is the first report that a high acyl/free carnitine ratio is a risk factor for post-pancreatectomy steatosis. Carnitine has an essential role in lipid metabolism as a transport of fatty acid from cytosol to mitochondria [17,18]. Fatty acid is conjugated with CoA and forms acyl-CoA, which is conjugated with carnitine and forms acylcarnitine. Acylcarnitine is translocated into matrix through the inner mitochondria membrane for subsequent beta oxidation, which is one of the primary pathways of lipids metabolism [19,20]. Therefore, total carnitine deficiency or increase ratio of acyl/free carnitine ratio has been reported to reflect the mitochondrial dysfunction causing inhibition of fatty acid beta oxidation and disorder in lipid metabolisms [17,20], which may cause hepatic steatosis.

We also found that more than half of our post-pancreatectomy patients had hypocarnitinemia. However, carnitine concentrations did not correlate with the presence of hepatic steatosis in our study. A relationship between carnitine concentrations and hepatic steatosis has not previously been reported and the small size of this study prevents us from drawing definite conclusions about the relationship between carnitine concentration and hepatic steatosis. We have recently reported that L-carnicine treatment improves post-pancreatectomy hepatic steatosis [15]. Malaguarnera et al. have also reported that L-carnicine treatment improves NASH and suggested that L-carnitine may have beneficial effects on the mitochondrial respiratory chain [21]. Ishikawa et al. reported that L-carnitine prevents progression of NASH in a mouse model by upregulating beta-oxidation [22]. These findings suggest that low carnitine concentrations may induce mitochondrial dysfunction, leading in turn to development of hepatic steatosis.

The limitations of this study include the small sample size, lack of data on preoperative carnitine concentrations, and the retrospective nature of the study. We emphasise that this study is not a validation study but an exploratory one. We are now planning a prospective, randomized, controlled study in which we will monitor carnitine concentrations both before and after pancreatectomy with the aim of conclusively identifying the effect of L-carnitine on hepatic steatosis in post-pancreatectomy patients.

In conclusion, we here reported that the acyl/free carnitine ratio is a risk factor for hepatic steatosis after pancreatectomy. Monitoring the acyl/free carnitine ratio and L-carnitine supplementation may prevent hepatic steatosis after pancreatectomy. The significance of our findings is not restricted to the field of pancreatic surgery. Hepatic steatosis and NASH with malnutrition is a serious and common problem in patients with diseases causing malnutrition, typically anorexia nervosa, with which approximately 30% of patients show liver dysfunction [23–25] and occasionally develop acute hepatic failure [26]. Currently, no established therapy is available in such cases [23]. Our findings surely shed a light to these miserable situations in broader field.

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