What are the Risk Factors for Non-Alcoholic Fatty Liver Disease after Pancreatoduodenectomy?

Mitsugi Shimoda¹, Takayuki Shimizu², Yoshimi Iwasaki², Masato Kato², Keiichi Kubota²

Corresponding author:

Mitsugi Shimoda, M.D., Ph.D.

3-20-1, Chuo, Ami, Ibaraki

300-0395, Japan

Tel: +81-29-887-1161

Fax: +81-29-840-2089

E-mail: mshimoda@tokyo-med.ac.jp

¹Department of Gastroenterological Surgery, Tokyo Medical University, Ibaraki Medical Center

²Second Department of Surgery, Dokkyo Medical University, Japan

ABSTRACT

Abbreviations:

PD - Pancreatoduodenectomy;

NAFLD - Non-Alcoholic Fatty Liver Disease;

Fe - Iron;

Zn - Zinc;

Cu - copper;

SSPPD - Subtotal Stomach-Preserving PD;

HU - Hounsfield Unit;

DGE - Delayed Gastric Emptying;

PF - Pancreatic Fistula;

PJ - Pancreatojejunostomy;

CJ - Choledochojejunostomy;

ROC - Receiver Operating Characteristic

Background: Although hepatic steatosis is often observed after pancreatoduodenectomy (PD), its preoperative clinical features remain unclear. This study focused on the risk factors for non-alcoholic fatty liver disease (NAFLD) after PD.

Methodology: Between April 2008 and June 2011, 100 patients underwent subtotal stomach-preserving PD (SSPPD) for pancreatic head or periampullary diseases. We divided the patients into two groups: a NAFLD group (NA group) and a non-NAFLD group (non-NA group). We compared risk factors such as BMI, blood parameters, blood cell count and trace elements (Fe, Zn, Cu and pre-albumin) in the serum between the two groups. NAFLD was defined as being present when the HU value was less than 40 or when the spleen/liver HU ratio was more than 1.5 on the basis of abdominal CT three months after surgery.

Results: Twenty-seven of the 100 (27.0%) patients had NAFLD within three months after surgery. Univariate analysis selected age, reconstruction for Gastrojejunostomy, the pre-operative Alb, AST, ALT, Fe, and Zn levels as risk factors for NAFLD. At multivariate analyses, only preoperative Fe level was a risk factor for NAFLD (odd ratio 1.052; 95% C.I. 1.457-29.663; $p=0.034$).

Conclusions: Preoperative serum Fe level is the only independent risk factor for NAFLD after PD.

Key words: pancreatoduodenectomy (PD), iron (Fe), zinc (Zn), non-alcoholic fatty liver disease (NAFLD)

INTRODUCTION

Pancreaticoduodenectomy (PD) is a standard surgical treatment for patients with pancreatic or periampullary tumors. In the last decade, PD has become a safely performed procedure in high-volume surgical institutions. With improvements in operative techniques, high-quality perioperative management, and advances in modalities for patient selection, the postoperative mortality rate has decreased to less than 5% (1, 2). However, during the postoperative course, a proportion of patients show poor food intake or weight loss, and some develop non-alcoholic fatty liver disease (NAFLD). Previous reports have indicated that NAFLD occurs in 23-37% of patients after PD, and that its risk factors include the volume of resected pancreas, postoperative diarrhoea, lack of hyperlipidaemia,

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and insulin resistance (3, 4). On the other hand, some previous studies have suggested that trace elements such as iron (Fe), zinc (Zn) and copper (Cu) might facilitate the development of NAFLD (5, 6). However, the role of serum trace elements in the development of NAFLD after PD has remained unclear.

In this retrospective study of 100 consecutive PDs, we evaluated the influence of pre- and postoperative factors, operative technique, and commonly accepted risk factors on the incidence of NAFLD, focusing particularly on trace elements in the serum such as iron (Fe), zinc (Zn), copper (Cu), and prealbumin.

MATERIAL AND METHOD

Patients

Patients who underwent subtotal stomach-preserving PD (SSPPD) for pancreatic head and periampullary tumors at our hospital between April 2008 and June 2011 were evaluated in this study. One patient who had NAFLD before surgery, and two with a history of alcohol abuse were excluded. A total of 100 patients were enrolled. All clinical, pathological and biochemical data were collected, and analyzed retrospectively. Informed consent was obtained from all of 100 patients in this retrospective study. In our analysis, we compared data between the NAFLD group (NA group) and non-NAFLD group (non-NA group).

Preoperative evaluation

Preoperative evaluations included physical examination, routine laboratory tests, and contrast-enhanced computed tomography (CT). To be considered for resection, patients had to fulfil the following objective radiographic criteria: 1) absence of metastatic lesions; 2) no evidence of tumour involvement of the superior mesenteric artery or celiac axis. Biliary drainage was performed in patients with jaundice via the percutaneous trans-hepatic or naso-biliary route (PTBD or ENBD) in order to reduce the serum bilirubin level to less than 5 g/dl.

Postoperative management

For at least 7-10 days after surgery, all patients received total parenteral nutrition (TPN) along with a trace element solution at 2 ml/day (Fe: 4.73 mg/ml, Zn: 8.625 mg/ml, Cu: 0.624 mg/ml, manganese (Mn): 0.09895 mg/ml) and a proton pump inhibitor (PPI) or histamine H₂-receptor antagonist. The nasogastric tube (NGT) was left in place until postoperative day (POD) 5 to 7. We performed an upper GI series in all cases. If the patients did not show leakage or stenosis of the anasto-

mosis, oral intake was started with water or tea and progressed in multiple steps every 1-2 days to a solid diet. However, the NGT was reinserted in cases of vomiting or nausea. Pancreatic enzymes (pancreatin 3 g/day: Yoshida Pharmaceutical Co. Ltd, Tokyo, Japan) were given to all patients and continued for 1 year after surgery.

Blood samples

Blood samples were obtained preoperatively (Pre), on POD 7 and 21, and 3 months after the operation, respectively. Blood chemical measurements included total protein (TP, g/dl), albumin (Alb, g/dl), prealbumin (Pre-Alb, mg/dl), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), γ -glutamyl transpeptidase (γ -GTP, IU/l), alkaline phosphatase (ALP, IU/l), blood urea nitrogen (BUN, mg/dl), creatinine (Cr, mg/dl), blood sugar (BS, g/dl), electrolytes (Na, K and Cl, mEq/l), blood cell counts (WBC: $\times 10^3/\mu\text{l}$, RBC: $\times 10^6/\mu\text{l}$, hemoglobin: Hb, g/dl, platelets: Plt, $\times 10^3/\mu\text{l}$, neutrophils: Neu, %), trace elements (Fe, Zn, Cu: $\mu\text{g/dl}$), and C-reactive protein (CRP, mg/dl).

Definition of postoperative complications

NAFLD was defined on the basis of a median Hounsfield unit (HU) value of the liver (CT value) not exceeding 40 HU (7, 8), or a spleen/liver HU ratio of more than 1.5, determined by abdominal plain CT before, and three months after surgery. The median HU value of the liver and spleen parenchyma was calculated using a 3-point scale in the same abdominal CT slice.

Delayed gastric emptying (DGE) was classified into three grades (A, B and C) based on clinical impact, according to the definition proposed by the International Study Group of Pancreatic Surgery (ISGPS) (9).

Pancreatic fistula (PF) is characterized by amylase values in drained fluid greater than three times the upper limit of normal serum amylase values measured on POD 3. Three grades of PF (A, B, and C) were defined according to the standards set by the International Study Group of Pancreatic Fistula (10). Grade B and C patients were also evaluated in this series.

Surgery

PD was performed using the standard method. Anastomotic reconstruction was performed by the modified Child method described elsewhere (11). The pancreas division line was on the left side of the portal vein in all cases. SSPPD included the division of the stomach 2 cm proximal to the pyloric ring and the resection of the entire duodenum distal to the transection site, together with the gallbladder, distal

common bile duct, and pancreatic head, as well as en bloc resection of the lymph nodes. If the tumour had invaded into the portal vein or superior mesenteric vein, the vein was resected and repaired to achieve curative surgery.

First, pancreaticojejunostomy (PJ) after SSPPD was performed by the Kakita method (12), using an external pancreatic duct stent (4-10 Fr polyethylene pancreatic drainage tube with a small knob: Sumitomo Bakelite Co., Japan) and duct-to-mucosa anastomosis. All stitches were done with monofilament non-absorbable suture (4-0 Prolene using the Kakita method and/or 5-0 or 6-0 Prolene for duct-to-mucosa sutures). Then, an end-to-side choledochojejunostomy (CJ) with an internal stent (short stent: 3 cm in length, 4-10 Fr polyethylene pancreatic drainage tube with a small knob: Sumitomo Bakelite Co., Japan) was performed. After PJ and CJ, the gastrojejunostomy (GJ) was performed. A jejunal loop about 40 cm distal to the CJ was brought up anteriorly to the transverse colon and anastomosed to the stomach. The GJ was constructed by end-to-side two-layer Billroth-II (B-II) or Roux-en-Y (R-Y) reconstruction. After the procedure, double drains were placed at the PJ, CJ and Winslow foramen.

Statistical analysis

Multivariate analysis was performed using variables that had been selected by univariate analysis on the basis of a cut-off probability value of $p < 0.05$. The recommended cut-off values were defined by receiver operating characteristic (ROC) curve analysis. To determine the optimal cut-off points, the Youden index method was used (13, 14).

Differences between groups were analyzed using the chi-squared test. Odds ratios with 95% confidence interval (95% CI) were calculated using univariate or multivariate analysis. Statistical analyses were performed using the SPSS statistical software package, version 16.0 (SPSS Inc., Chicago, IL) at a significance level of $p < 0.05$.

RESULTS

Incidence of NAFLD

The incidence of postoperative NAFLD was 27.0% (27/100). There were 25 patients whose CT values were less than 40 HU, while the remaining 2 patients had CT values exceeding 40 HU but spleen/liver HU ratios exceeding 1.5. In 14 of these 27 patients, the spleen/liver HU ratios were more than 1.5.

The pre- and postoperative liver CT values in the NA group were 52.8 ± 8.25 and 25.7 ± 12.3 , respectively, showing a significant decrease in the postoperative value ($p = 0.0001$). There were no significant differences in the pre- and postoperative spleen CT values between the two groups, and there was no difference in the liver CT value before and after surgery in the non-NA group (fig. 1).

Risk factors for pre- and postoperative NAFLD

Univariate analysis identified 7 factors that were associated with the development of NAFLD: age, R-Y reconstruction, serum Alb, AST, ALT, Fe and Zn levels at Preoperative, serum pre-alb, γ -GTP, Fe and Zn levels at POD 7, serum AST, ALT, γ -GTP and Hb levels at POD 21, and serum AST, ALT and γ -GTP levels at 3 months after

Figure 1 - Pre-and post-operative CT value in liver and spleen

Table 1 - Univariate analysis of preoperative factors related to NAFLD after PD

	NA group (n=27)	Non-NA group (n=73)	P-value	Odds ratio	95%C.I.
Gender (M/F)	14/13	36/37	0.150	1.935	0.778-4.750
Age (yr)	60.1 (40.3-78.5)	70.0 (39.2-84.6)	0.004	0.934	0.892-0.978
Reconstruction (B-II/R-Y)	8/19	37/36	0.043	0.378	0.147-0.971
Bleeding (ml)	600 (280-2930)	612.5 (222-2911)	0.938	1.000	0.147-0.971
Op. time (hr:min)	7:55 (5:50-11:49)	8:02 (6:00-10:49)	0.730	1.001	0.995-1.007
Malignant disease (yes/no)	25/2	66/7	0.749	1.306	0.254-6.714
BMI	21.6 (12.5-31.1)	21.1 (16.9-31.1)	0.596	0.972	0.876-1.079
T.P (g/dl)	6.6 (5.7-7.8)	6.6 (4.7-8.6)	0.603	1.200	0.603-2.387
Alb (g/dl)	3.6 (2.4-4.3)	3.3 (2.2-4.5)	0.080	2.216	0.909-5.402
Pre alb (mg/dl)	23.3 (8.8-37.3)	22.5 (8.5-36.0)	0.069	1.023	0.998-1.047
BS (mg/dl)	100 (50-191)	94 (60-294)	0.445	1.004	0.993-1.015
AST (IU/L)	32 (12-141)	25 (6-176)	0.557	1.004	0.990-1.019
ALT (IU/L)	43 (10-325)	36 (3-231)	0.075	1.007	0.999-1.015
γ -GTP (IU/L)	142 (17-898)	82 (10-768)	0.100	1.002	1.000-1.005
ALP (IU/L)	367 (149-1283)	383 (142-2038)	0.936	1.000	0.949-1.001
BUN (mg/dl)	11 (6-18)	13 (5-37)	0.115	0.910	0.819-1.023
Cr (mg/dl)	0.65 (1.49)	0.705 (0.4-1.45)	0.222	0.286	0.038-2.133
Na (mEq/l)	141 (134-147)	140 (131-147)	0.310	1.080	0.931-1.253
K (mEq/l)	4.2 (3.6-5.2)	4.2 (2.6-5.4)	0.244	1.735	0.687-4.382
Cl (mEq/l)	105 (98-109)	105 (93-114)	0.880	1.011	0.882-1.158
WBC ($\times 10^3/\mu$ l)	5.0 (2.1-8.4)	6.05 (2.1-5.5)	0.027	1.000	0.999-1.000
Neutro (%)	57.3 (39.3-76.1)	61.9 (32.2-88.6)	0.226	0.975	0.935-1.016
RBC ($\times 10^6/\mu$ l)	4.25 (3.12-5.18)	4.18 (3.95-4.98)	0.225	0.915	0.812-1.028
Hb (g/dl)	12.3 (8.3-17.0)	12.3 (8.1-15.9)	0.258	1.145	0.906-1.448
Plt ($\times 10^3/\mu$ l)	21.9 (13.2-32.7)	21.0 (8.8-68.7)	0.716	0.954	0.894-1.018
CRP (mg/dl)	0.1 (0.1-2.84)	0.16 (0.1-13.8)	0.195	0.496	0.172-1.432
Fe (μ g/dl)	85 (26-176)	63 (16-146)	0.006	1.024	1.007-1.042
Zn (μ g/dl)	86 (53-130)	71.5 (40-114)	0.011	1.037	1.009-1.067
Cu (μ g/dl)	105 (79-160)	101.5 (17-154)	0.479	1.007	0.988-1.025

Median (range), body mass index: BMI, total protein: TP, Albumin: Alb, blood sugar: BS, creatinine: Cr, hemoglobin: Hb, platelet: Plt, C-reactive protein: CRP, iron: Fe, zinc: Zn, copper: Cu, postoperative days: POD

surgery (tables 1-4). As far as complications were concerned, PF and DGE were not associated risk factors for NAFLD (table 5).

After multivariate analysis was performed (table 6), only one factor remained significant. Fe ($p=0.034$) was selected as the most potent preoperative risk factor for NAFLD.

For Fe at Pre, ROC curve analysis revealed that 73.5 μ g/dl was an appropriate cut-off value for detection of NAFLD preoperatively (sensitivity: 64.0%, specificity: 70.5%).

Most prominent point disclosed two parameters such as sensitivity (0.640) and 1-specificity (0.705) (fig. 2).

DISCUSSION

NAFLD includes a spectrum of hepatic histological

features ranging from hepatic steatosis to inflammatory changes with or without fibrosis, referred to as non-alcoholic steatohepatitis (NASH). NAFLD is particularly common among overweight and obese individuals (15, 16). NASH may progress to advanced fibrosis and cirrhosis in a significant proportion of those affected (17). However, its exact prevalence and pathophysiology are still unclear. Several factors including oxidative stress, iron overload, and altered lipid metabolism are reportedly associated with the progression of simple steatosis to NASH (5, 18). Overall, a strong association has been observed between NAFLD and insulin resistance, leading to the suggestion that NAFLD may be the hepatic manifestation of metabolic syndrome (19). Several factors, such as liver injury mediated by endotoxins or cytokines and oxidative stress, have been postulated. These are sometimes induced by major

Table 2 - Univariate analysis of factors related to NAFLD after PD at POD 7

	NA group (n=27)	Non-NA group (n=73)	P-value	Odds ratio	95%C.I.
BMI	20.2 (13.0-31.8)	20.0 (15.9-29.1)	0.419	0.974	0.913-1.038
T.P (g/dl)	5.8 (4.0-7.2)	6.0 (4.2-7.9)	0.169	0.666	0.374-1.188
Alb (g/dl)	2.5 (1.9-3.4)	2.6 (1.6-3.6)	0.738	1.214	0.389-3.788
Pre alb (mg/dl)	11.8 (5.9-27.8)	11.4 (3.5-28.5)	0.468	1.034	0.945-1.132
BS (mg/dl)	98 (61-139)	103.5 (61-373)	0.097	0.986	0.969-1.003
AST (IU/L)	29 (10-122)	23 (8-101)	0.145	1.017	0.994-1.041
ALT (IU/L)	31 (10-155)	26 (7-320)	0.388	1.004	0.995-1.014
γ -GTP (IU/L)	52 (22-262)	42 (10-187)	0.158	1.007	0.997-1.016
ALP (IU/L)	464 (110-948)	333 (172-1369)	0.395	1.001	0.999-1.002
BUN (mg/dl)	9.5 (4-29)	11 (5-44)	0.187	0.940	0.857-1.031
Cr (mg/dl)	0.67 (0.33-1.16)	0.66 (0.28-1.43)	0.361	0.931	0.971-2.618
Na (mEq/l)	138 (130-145)	136 (128-151)	0.257	1.068	0.953-1.197
K (mEq/l)	4.4 (3.5-4.9)	4.4 (3.2-5.6)	0.687	0.964	0.805-1.153
Cl (mEq/l)	99 (93-105)	99 (89-110)	0.567	1.035	0.921-1.162
WBC ($\times 10^3/\mu\text{l}$)	8.8 (3.0-12.6)	9.35 (3.2-19.9)	0.048	1.000	1.000-1.000
Neutro (%)	76.3 (55.3-87.6)	76.4 (53.4-92.7)	0.284	0.968	0.912-1.027
RBC ($\times 10^3/\mu\text{l}$)	4.06 (3.11-4.49)	3.97 (2.98-4.38)	0.568	1.036	0.921-1.089
Hb (g/dl)	10.8 (8.1-15.8)	10.9 (7.4-14.5)	0.753	1.046	0.792-1.381
Plt ($\times 10^3/\mu\text{l}$)	23.5 (11.7-40.2)	24.8 (10.6-56.0)	0.154	0.954	0.894-1.018
CRP (mg/dl)	2.18 (0.1-13.95)	4.7 (0.18-20.72)	0.263	0.932	0.837-1.038
Fe ($\mu\text{g/dl}$)	29 (18-148)	25 (11-82)	0.012	1.054	1.012-1.099
Zn ($\mu\text{g/dl}$)	74 (43-118)	67.5 (36-100)	0.036	1.033	1.002-1.065
Cu ($\mu\text{g/dl}$)	87 (57-126)	92 (30-143)	0.510	0.992	0.970-1.015

Median (range), body mass index: BMI, total protein: TP, Albumin: Alb, blood sugar: BS, creatinine: Cr, hemoglobin: Hb, platelet: Plt, C-reactive protein: CRP, iron: Fe, zinc: Zn, copper: Cu, postoperative days: POD

abdominal surgery. A previous report has documented that NAFLD was observed in 37% of the patients who underwent PD, and that the risk factors for NAFLD after PD were pancreatic cancer, the pancreatic resection line and postoperative diarrhoea (3). Another study found that 23% of patients developed NAFLD after PD, and that the only evident associated risk factor was pancreatic cancer (4). All of these risk factors become clear after the operation.

In the present study, for the first time, we investigated the pre- and postoperative risk factors for NAFLD after PD, and found that the preoperative serum level of Fe was significantly associated with this complication. In our series, NAFLD developed in 27% of patients who underwent PD, being a proportion which is comparable with a previously reported figure (3). In the present NA group, there were significant differences in the liver CT values at Pre and 3 months after PD, suggesting that CT

Figure 2 - ROC curve in Fe

Table 3 - Univariate analysis of factors related to NAFLD after PD at POD 21

	NA group (n=27)	Non-NA group (n=73)	P-value	Odds ratio	95%C.I.
BMI	21.1 (12.9-32.4)	20.6 (16.6-30.5)	0.983	0.999	0.940-1.063
T.P (g/dl)	5.1 (4.4-6.1)	5.2 (4.0-6.6)	0.112	0.489	0.203-1.181
Alb (g/dl)	2.2 (1.7-3.0)	2.2 (1.6-3.5)	0.359	1.211	0.804-1.824
Pre alb (mg/dl)	12.7 (6.7-19.4)	10.9 (4.0-23.5)	0.074	1.123	0.989-1.275
BS (mg/dl)	149 (86-265)	128 (62-332)	0.769	0.999	0.991-1.007
AST (IU/L)	36 (15-174)	36 (11-185)	0.721	1.002	0.991-1.014
ALT (IU/L)	72 (17-371)	65 (10-317)	0.259	1.003	0.997-1.010
γ -GTP (IU/L)	128 (28-320)	96 (13-472)	0.238	1.003	0.998-1.008
ALP (IU/L)	445 (154-1361)	387 (147-2361)	0.991	1.000	0.999-1.001
BUN (mg/dl)	12 (5-31)	12 (6-38)	0.412	0.996	0.891-1.049
Cr (mg/dl)	0.58 (0.28-2.18)	0.61 (0.32-2.18)	0.262	0.285	0.032-2.553
Na (mEq/l)	136 (126-143)	135 (123-143)	0.271	1.076	0.945-1.225
K (mEq/l)	4.4 (3.6-5.4)	4.2 (2.5-6)	0.109	1.855	0.871-3.947
Cl (mEq/l)	102 (91-109)	101 (92-110)	0.264	0.968	0.915-1.025
WBC ($\times 10^3/\mu l$)	5.8 (3.1-11.1)	6.9 (3.0-24.5)	0.112	1.000	1.000-1.000
Neutro (%)	63.4 (47.8-80.7)	68.1 (35.0-94.8)	0.278	0.980	0.944-1.017
RBC ($\times 10^6/\mu l$)	4.18 (3.10-4.38)	4.02 (2.81-4.32)	0.120	1.015	1.014-1.181
Hb (g/dl)	11.5 (7.8-14.6)	10.5 (7.4-14.9)	0.041	1.355	1.012-1.814
Plt ($\times 10^3/\mu l$)	29.2 (15.4-51.8)	30.8 (10.1-69.3)	0.665	0.995	0.944-1.037
CRP (mg/dl)	0.89 (0.1-13.95)	1.22 (0.1-11.66)	0.581	0.951	0.797-1.135
Fe ($\mu g/dl$)	29 (8-87)	28.5 (10-106)	0.415	1.011	0.985-1.038
Zn ($\mu g/dl$)	91.5 (72-126)	97 (39-133)	0.451	1.011	0.983-1.040
Cu ($\mu g/dl$)	100 (46-131)	104 (17-180)	0.741	0.997	0.977-1.016

Median (range), body mass index: BMI, total protein: TP, Albumin: Alb, blood sugar: BS, creatinine: Cr, haemoglobin: Hb, platelet: Plt, C-reactive protein: CRP, iron: Fe, zinc: Zn, copper: Cu, postoperative days: POD

Table 4 - Univariate analysis of factors related to NAFLD after PD at 3 months after surgery

	NA group (n=27)	Non-NA group (n=73)	P-value	Odds ratio	95%C.I.
BMI	19.7 (13-30.9)	18.9 (15-27.6)	0.890	0.995	0.926-1.069
T.P (g/dl)	6.3 (4.9-7.3)	6.5 (3.6-7.9)	0.786	0.923	0.519-1.643
Alb (g/dl)	3.1 (2.1-4.4)	3.0 (1.6-4.3)	0.405	1.416	0.624-3.213
Pre alb (mg/dl)	13.6 (5.7-26.4)	13.4 (3.8-29.9)	0.778	0.987	0.896-1.087
BS (mg/dl)	105 (73-371)	111 (50-421)	0.297	0.996	0.988-1.004
AST (IU/L)	33 (11-110)	23 (6-85)	0.018	1.033	1.006-1.062
ALT (IU/L)	36 (12-127)	19 (12-214)	0.102	1.014	0.997-1.031
γ -GTP (IU/L)	36 (14-243)	26 (10-94)	0.121	1.018	0.995-1.040
ALP (IU/L)	377 (139-872)	292 (169-880)	0.058	1.004	1.000-1.008
BUN (mg/dl)	11 (7-20)	12 (5-62)	0.111	0.903	0.797-1.024
Cr (mg/dl)	0.63 (0.31-1.1)	0.63 (0.31-1.62)	0.287	0.268	0.224-3.021
Na (mEq/l)	141 (134-144)	139 (127-144)	0.023	1.274	1.033-1.571
K (mEq/l)	4.3 (3.2-5.5)	4.3 (2.8-5.8)	0.888	1.060	0.471-2.387
Cl (mEq/l)	105 (96-116)	104 (90-113)	0.155	1.091	0.968-1.229
WBC ($\times 10^3/\mu l$)	5.3 (2.1-11.1)	4.9 (1.5-19.2)	0.629	1.000	1.000-1.000
Neutro (%)	59.7 (29.9-93.6)	56.1 (24.4-92.6)	0.205	1.021	0.989-1.054
RBC ($\times 10^6/\mu l$)	4.20 (4.10-4.67)	3.98 (2.87-4.76)	0.564	1.010	0.814-1.168
Hb (g/dl)	11.0 (8.3-13.4)	11.0 (6.6-14.8)	0.465	1.133	0.811-1.582
Plt ($\times 10^3/\mu l$)	24.5 (12.2-52.0)	20.5 (3.5-61.3)	0.239	1.030	0.981-1.082
CRP (mg/dl)	0.16 (0.1-12.21)	0.25 (0.1-11.66)	0.491	1.066	0.888-1.281
Fe ($\mu g/dl$)	57 (26-124)	54 (14-114)	0.381	1.014	0.983-1.046
Zn ($\mu g/dl$)	73.5 (35-125)	68 (29-128)	0.501	1.008	0.985-1.030
Cu ($\mu g/dl$)	86.5 (51-129)	86.0 (17-140)	0.846	0.998	0.976-1.020

Median (range), body mass index: BMI, total protein: TP, Albumin: Alb, blood sugar: BS, creatinine: Cr, haemoglobin: Hb, platelet: Plt, C-reactive protein: CRP, iron: Fe, zinc: Zn, copper: Cu, postoperative days: POD

Table 5 - Univariate analysis of postoperative complications related to NAFLD after PD

	NA group (n=27)	Non-NA group (n=73)	P-value	Odds ratio	95%C.I.
PF (yes/no)	6/21	17/56	0.632	0.762	0.251-2.317
DGE (yes/no)	4/23	17/56	0.375	0.583	0.177-1.921

Pancreatic fistula: PF, Delayed gastric emptying: DGE

Table 6 - Multivariate analysis of selected clinical risk factors for NAFLD after PD

	P-value	Odds ratio	95%C.I.
age	0.061	0.923	0.849-1.004
R-Y reconstruction	0.223	0.359	0.069-1.863
AST after 3 months	0.027	1.053	1.006-1.103
Hb at 21 POD	0.115	1.225	0.952-1.578
Preop.Fe	0.034	1.052	1.007-1.041
Preop.Zn	0.030	1.062	1.006-1.121
Fe at 7 POD	0.026	1.100	1.012-1.197
Zn at 7 POD	0.223	0.966	0.914-1.021

Hemoglobin: Hb, iron: Fe, zinc: Zn, copper: Cu, preoperation: preope, postoperative days: POD

at 3 months after PD is advisable to screen for NAFLD. The preoperative AST and ALT levels in the NA group were significantly higher compared to the non NA group. This result would suggest that preoperative liver function seems to become a risk factor for NAFLD post PD. However, no significant difference was observed at multivariate analysis. As a matter of fact, multivariate analysis revealed that the preoperative serum Fe level, was the only significant risk factor for NAFLD. Fe has also been extensively investigated in relation to liver injury (19, 20). Bonkovsky et al. reported that the prevalence of hemochromatosis protein gene (HFE) mutations associated with hereditary hemochromatosis was higher among patients with NASH and NAFLD (5). A significantly higher prevalence of HFE mutations in NAFLD patients has been reported to be a factor responsible for liver fibrosis by increasing hepatic iron deposition (21), but recent studies have failed to confirm this (22). Kosuge et al. compared the level of Fe with those of CRP, IL-6 and hepcidin in PD patients before and after surgery, and found that Fe was dependent on the expression of the latter three factors. Surgical stress induces inflammation, as expressed by an increase of CRP and IL-6, resulting in an increase of hepcidin production, which in turn suppresses the release of iron from macrophages at POD 3. The Fe level is suppressed thereafter (23).

In a clinical setting, it would be useful to predict the development of postoperative NAFLD before surgery.

Table 7- Analysis of patients in the NA and non-NA groups using selected cut-off values

	NA group (n=27)	Non-NA group (n=63)	P-value
Fe ($\geq 73.5 \mu\text{g/dl}$ / $< 73.5 \mu\text{g/dl}$)	21/6	16/57	0.0001
Zn ($\geq 78.5 \mu\text{g/dl}$ / $< 78.5 \mu\text{g/dl}$)	16/11	24/49	0.016

For this purpose, the preoperative cut-off values of Fe postulated in the present study would be helpful. Preoperative Fe values of $73.5 \mu\text{g/dl}$, efficiently discriminated patients who would develop NAFLD after PD from those who wouldn't. Patients who have a Fe level above this cut-off values should be recognized as a high-risk group for NAFLD after PD.

One interesting question is whether or not post-operative NAFLD could be avoided by reduction of the preoperative Fe level. To answer this question, a prospective trial of aggressive preoperative control of Fe will be necessary.

CONCLUSION

NAFLD after PD was observed in 27% of the patients in our study. Patients who have preoperative Fe value exceeding $73.5 \mu\text{g/dl}$ should be carefully monitored for the development of NAFLD after PD.

Conflict of interests

The authors declare no conflict of interests.

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