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ORIGINAL ARTICLE

Pancreatectomy Using the No-Touch Isolation Technique Followed by Extensive Intraoperative Peritoneal Lavage to Prevent Cancer Cell Dissemination: A Pilot Study

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ABSTRACT

Context In pancreatic cancer, even for patients who have undergone curative resection (R0), survival analysis has revealed a poor survival rate due to cancer recurrence. Because the operation itself might have caused the dissemination of these cancer cells, the no-touch isolation technique and extensive intraoperative peritoneal lavage may be a potential operative procedure for improving the outcome.

Patients Eight patients treated by the no-touch isolation technique were compared with 10 patients treated using conventional techniques.

Main outcome measures Cancer cell detection rates in the portal venous blood, frequency of recurrence, and survival rate. We also analyzed the lymphatic fluid squeezed from the resected cancerous pancreatic tissue.

Results In 5 out of 10 cases (50%) in the conventional procedure group, CEA mRNA was identified in the portal blood after tumor manipulation, while only 1 out of 8 cases (13%) in the no-touch isolation technique group was positive for portal CEA mRNA. All lymphatic fluid samples squeezed from the resected cancerous pancreatic tissue were positive (8/8) for CEA mRNA. The recurrence rate was 90% (9/10) in the conventional procedure group, and 38% (3/8) in the no-touch isolation technique group (P=0.043). In the conventional procedure group, hepatic metastasis, local recurrence, peritoneal dissemination, and extraabdominal recurrence were identified in 6 (60%), 4 (40%), 4 (40%), and 2 patients (20%), respectively. On the other hand, among the no-touch isolation technique group, recurrence was identified in 1 (13%), 1 (13%), 0 (0%), and 1 patient (13%), respectively. There was no peritoneal dissemination along with the decreased hepatic recurrence rate. Mean (±SEM) survival time was 21.2±5.8 months for the conventional procedure group and 41.5±5.6 months for the no-touch isolation technique group (P=0.018). The 3-year survival rate was 12.5±11.5% for the conventional procedure group and 75.0±21.7% for the no-touch isolation technique group.

Conclusion This study presented the potential of cancer dissemination during the intraoperative manipulation of tumors and its contribution to cancer recurrence, as well as the significance of the no-touch isolation technique and extensive intraoperative peritoneal lavage for pancreatic cancer surgery.

INTRODUCTION

Despite the development of new and different surgical procedures, hepatic metastases, local recurrences, and peritoneal dissemination are still major problems in the treatment of pancreatic cancer. For the majority of patients, pancreatic cancer is a systemic disease. Even for patients who have undergone curative resection (R0), survival analysis has revealed a poor survival rate due to cancer recurrence [1]. The majority of post-operative recurrences are due to hepatic metastasis, local recurrence, and peritoneal dissemination [2, 3, 4, 5]. As a recurrence mechanism, it is thought that minimal cancer cells already existed at the site of the recurrence (micrometastasis) at the time of surgery [6, 7, 8, 9, 10]. However, the operation itself might have caused the dissemination of these cancer cells.

Surgeons usually grasp the tumor during pancreatectomy before dissection of the surrounding vessels as shown in Figure 1. This procedure may increase the risk of squeezing and shedding the cancer cells into the portal vein, retroperitoneum, and/or peritoneal cavity while handling the tumor. The no-touch isolation technique (NTIT) is a procedure originally advocated as a strategy to protect cancer cells from spreading as a result of handling malignant tumors during both colon and eye cancer surgery [11, 12]. NTIT may be a potential procedure, which might improve the results of pancreatic cancer surgery. We report on the use of NTIT in pancreatic cancer operations. We also compare cancer cell detection rates in the portal venous blood and the frequency of recurrence as well as the survival rate between NTIT and conventional techniques.

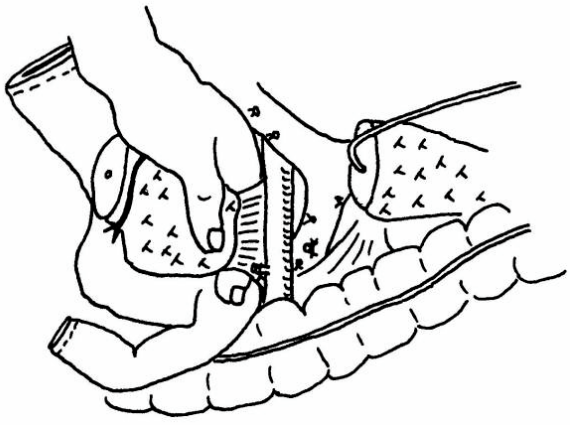

Figure 1. Pancreatic manipulation in conventional PD procedure. The pancreatic tissue containing cancer is grasped by surgeons before the dissection of the surrounding vessels during the conventional procedure.

PATIENTS AND METHODS

Patients

We performed a pancreatectomy in 18 cases of invasive pancreatic cancer (11 in the head of the pancreas and 7 in the body/tail region) between October 1999 and March 2004. During this period, we applied the conventional procedure of pancreatectomy to 10 cases (6 in the head of the pancreas and 4 in the body/tail region) and NTIT for 8 cases (5 head cancers and 3 body/tail cancers). The Japan Pancreas Society stage classification (I/II/III/IV) was 1/1/2/6 for the conventional group and 1/0/5/2 for the NTIT group. The International Union Against Cancer (UICC) stage classification (I/II/III/IV) was 1/1/5/3 for the conventional group and 1/4/2/1 for the NTIT group. Portal vein resection was performed in 4 cases in the conventional procedure group and in 4 cases in the NTIT group. Resection of the lymph nodes was in D2.

Study Design

We compared the cancer cell molecular detection rate frequency in the portal blood and in the lymphatic fluid squeezed from the cancer-containing excised tissue, as well as the frequency of recurrence (hepatic, local, peritoneal, and extraabdominal) (prospective non-randomized cohort study). Follow-up studies for the cancer recurrence were performed as follows: enhanced CT: every three months, measurement of serum tumor markers: every two months, physical examination: every month.

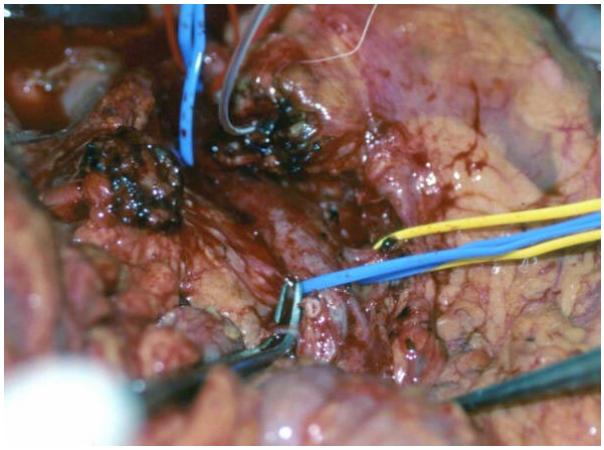

Figure 2. Exposure of the pancreatic veins in SSPPD using NTIT. The anterior inferior pancreatoduodenal veins are gently lifted using a Kelly forceps.

No-Touch Isolation Technique (NTIT)

The fundamental concept of NTIT is that the tumor must not be touched before the vascular and lymphatic drainage vessels are completely isolated.

A. Subtotal Stomach-Preserving Pancreatico-duodenectomy (SSPPD) for Carcinoma of the Pancreatic Head Region

After a laparotomy using an upper median incision and exploration of the peritoneal cavity, we first explored and ligated Henle's gastrocolic trunk vein at the communicating point to the superior mesenteric vein. Then, we divided the stomach (at the angle), pancreas, choledochus, and jejunum (about 5.0 cm from the Treitz's ligament), as well as the gastroduodenal, right gastric, and inferior pancreatic arteries. We ligated or sutured the cut ends of the pancreatic duct and choledochus to prevent dissemination. Thereafter, we ligated the portal vein branches, such as the posterior superior pancreatoduodenal vein, anterior inferior pancreatoduodenal vein, and posterior inferior pancreatoduodenal vein, to isolate the portal vein (Figure 2). We did not perform kocherization until the drainage vascular vessels were ligated. We then removed the pancreatic head from its posterior adhesion (kocherization), which resulted in removal of the pancreatic head, duodenum, gastric antrum, choledochus, and gallbladder. We ligated the cut end of the pancreatic and bile ducts (cancer side) and all lymphatic vessels and nerves to prevent any cancer cells from disseminating. This procedure was reported for pancreatic cancer by Hirota and Ogawa [13], and for periampullary cancer by Kobayashi *et al*. [14]. Finally, after the reconstruction, extensive intraoperative peritoneal lavage (EIPL) with 5-10 L of warm saline was performed to remove any disseminated cancer cells according to the modified method described by Shimada *et al*. [15].

When it was necessary to resect the portal vein, we first established a bypass between the iliac vein and the paraumbilical vein (or the great saphenous vein) using an antithrombogenic catheter, and then clamped the pancreatic venous flow with vascular clamps as proposed by Nakao and Takagi [16] (Figure 3). When dividing the portal vein, adequate absorption and rinsing are advisable because the portal blood might have free cancer cells present, which could leak into the abdominal cavity.

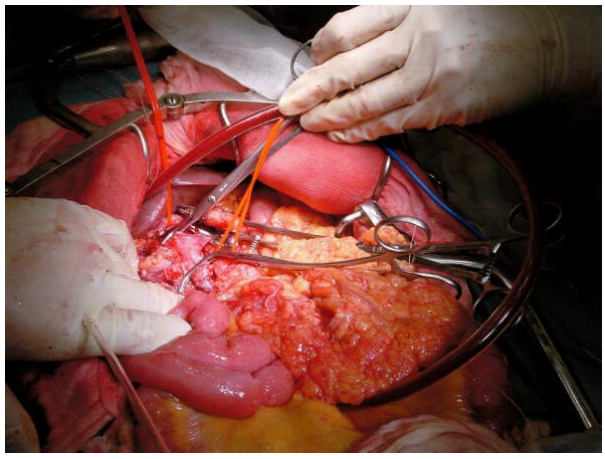

Figure 3. Management of a case which required resection of the portal vein. Accessible pancreatic veins were divided and a bypass was established between the iliac vein and the paraumbilical vein using an anti-thrombogenic portal vein bypass catheter. The portal vein was then clamped with vascular clamps. Since the portal venous flow was maintained via a catheter bypass, even after the clamping of the portal vein, the resection and anastomosis of the portal vein was performed safely and unhurriedly.

B. Distal Pancreatectomy (DP) for Carcinoma of the Pancreatic Body and Tail

After a laparotomy using an upper median plus left transverse incision (L-shaped) and exploration of the peritoneal cavity, we explored the middle colic and superior mesenteric veins and then divided the pancreas. This procedure allowed us to ligate the splenic vein and artery at the point where they diverge. Subsequently, we ligated the left gastroepiploic and short gastric vessels. Finally, we freed the pancreas from its posterior adhesion at the dorsal plane of Gerota's fascia to remove the pancreatic body and tail, and the spleen. The cut end of the pancreatic duct and lymphatic vessels and nerves were ligated to prevent the dissemination of cancer cells. After the procedure, EIPL was performed with 5-10 L of warm saline to remove any potential disseminated cancer cells.

Molecular Detection of Cancer Cells

Blood samples (10 mL) were obtained through a catheter in the portal vein before and during manipulation of the tumor. In some cases, the lymphatic fluid squeezed from the resected cancerous pancreatic tissue was collected. mRNA was extracted using the MagNA Pure LC system (Roche Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Real-time, one-step, no-nested RT-PCR for CEA mRNA was examined using a LightCycler (Roche Biochemicals, Mannheim, Germany) with the LightCycler RNA amplification kit for hybridization probes (Roche Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. In this system, the PCR is monitored using hybridization probes labeled with fluorescein (donor dye) or LC Red 640 (acceptor dye), allowing a fluorescence resonance energy transfer after hybridization to the target sequence in a head-to-tail arrangement on the same strand of the amplified DNA fragment. The intensity of the light emitted by LC Red 640 is proportional to the DNA formation and measured at 640 nm. In this analysis, the background fluorescence was removed by setting a noise band. We classified a sample as positive if the intensity of fluorescence exceeded the noise band.

The primer sequences used for CEA amplification were 5'-GACGCAAGAGCCTATGTATG and 5'-GGCATAGGTCCCGTTATTA. The probe sequences used for CEA identification were 5'-CCCAGACTCGTCTTACCTTTCGG-FL and 5' LC-AGCGAACCTCAACCTCTCCTGC-P. In the sequences, FL means fluorescein, LC means LC Red 640 labeling, and P means phosphate group to block the extension. All primers and probes were synthesized and purified using reverse-phase high performance liquid chromatography (Nihon Gene Research Laboratories, Sendai, Japan). After reverse transcription for 10 min at 50°C, the following temperature profile was used for amplification: denaturation for 1 cycle at 95°C for 30 sec and 45 cycles at 95°C for 1 sec, 55°C for 10 sec, and 72°C for 10 sec. Fluorescence was measured at the end of the annealing period of each cycle to monitor amplification. To verify the integrity of the isolated RNA, a PCR assay with primers and probes specific for the gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was conducted for each case under the same conditions as described above. The primer sequences used for GAPDH amplification were 5'-TGAACGGGAAGCTCACTGG and 5'-TCCACCACCCTGTTGCTGTA. The probe sequences used for GAPDH identification were 5'-TCAACAGCGACACCCACTCCT-FL and 5'-LC-CACCTTTGACGCTGGGGCT-P.

Each series of RT-PCR reactions included RNA-negative samples as a negative control. mRNA from WiDr cells, which is well known to express high amounts of CEA, was considered a positive control.

The people who performed the molecular detection of the cancer cells had not been informed of the operative procedure employed.

Table 1. Molecular detection rate of cancer cells in the portal venous blood and in the lymphatic fluid squeezed from the cancer tissue.

	Portal blood		Lymphatic fluid squeezed
	Before manipulation	During manipulation	from tissue
A. Conventional procedure group			
Head	0/6 (0%)	2/6 (33.3%)	2/2 (100%)
Body/Tail	0/4 (0%)	3/4 (75.0%)	2/2 (100%)
Total	**0/10 (0%)**	**5/10 (50.0%)**	**4/4 (100%)**
B. NTIT group			
Head	0/5 (0%)	1/5 (20.0%)	3/3 (100%)
Body/Tail	0/3 (0%)	0/3 (0%)	1/1 (100%)
Total	**0/8 (0%)**	**1/8 (12.5%)**	**4/4 (100%)**
P value (A *vs*. B)	1.000	0.152	1.000

ETHICS

This study was performed after obtaining the patients' written informed consent. The study protocol conformed to the Ethics Committee guidelines for Kumamoto University School of Medicine, and the ethical guidelines of the 1975 Declaration of Helsinki.

STATISTICS

Mean, SEM, 95% confidence intervals (95% CI) and frequencies were used as descriptive statistics. The Fisher's exact and the log-rank tests, as well as the Kaplan-Meier method, were applied. Two tailed P values less than 0.05 were considered statistically significant. Statistical analysis was performed by running the SPSS for Windows statistical package (Version 8) using a personal computer.

RESULTS

During the observation period between October 1999 and March 2004, in 5 out of 10 cases (50%) of the conventional procedure group, CEA mRNA was identified in the portal blood after tumor manipulation, while only 1 out of 8 cases (13%) was positive for portal CEA mRNA in the NTIT group; this difference did not reach statistical significance (P=0.152). All lymphatic fluid samples squeezed from the resected cancerous pancreatic tissue were positive (8/8) for CEA mRNA (Table 1).

Recurrence rate was 90% (9/10) in the conventional procedure group, and 38% (3/8) in the NTIT group (P=0.043; Table 2). Among the conventional procedure group, hepatic metastasis, local recurrence, peritoneal dissemination, and extraabdominal recurrence were identified in 6 (60%), 4 (40%), 4 (40%), and 2 patients (20%), respectively. On the other hand, among the NTIT group, recurrence was identified in 1 (13%), 1 (13%), 0 (0%), and 1 patient (13%), respectively. (Table 2). Total recurrence rates were significantly reduced in the NTIT group as compared to the conventional procedure group (P=0.043) while hepatic recurrence was

Table 2. Frequency of hepatic metastasis, local recurrence, and peritoneal dissemination among cases of pancreatic resection.

	Hepatic	Local	Peritoneal	Extra-abdominal	Total
A. Conventional procedure group					
Head	5/6 (83.3%)	2/6 (33.3%)	2/6 (33.3%)	0/6 (0%)	5/6 (83.3%)
Body/Tail	1/4 (25.0%)	2/4 (50.0%)	2/4 (50.0%)	2/4 (50.0%)	4/4 (100%)
Total	**6/10 (60.0%)**	**4/10 (40.0%)**	**4/10 (40.0%)**	**2/10 (20.0%)**	**9/10 (90.0%)**
B. NTIT group					
Head	1/5 (20.0%)	1/5 (20.0%)	0/5 (0%)	1/5 (20.0%)	3/5 (60.0%)
Body/Tail	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Total	**1/8 (12.5%)**	**1/8 (12.5%)**	**0/8 (0%)**	**1/8 (12.5%)**	**3/8 (37.5%)**
P value (A *vs*. B)	0.066	0.314	0.092	1.000	0.043

Table 3. Pattern of the recurrence in the NTIT group patients.

No.	Stage (JPS)	First operation	Recurrence site	Detected date	Subsequent treatment	Outcome
1.	III	SSPPD	Local	12 months after 1st operation	Gemcitabine	Died 23 months after1st operation
2.	III	SSPPD	Liver (single)	4 months after 1st operation	Hepatectomy I/low P*	Alive 16 months after 1st operation
3.	IVa	SSPPD	Lung (multiple)	13 months after 1st operation	I/low P	Alive 15 months after 1st operation

*Combined irinotecan and low-dose cisplatin therapy [28]

near statistical significance (P=0.066). The pattern of recurrence in the NTIT group is summarized in Table 3.

There was no operative mortality in either group. Mean survival time was 21.2±5.8 (95% CI: 9.9-32.5) months for the conventional procedure and 41.5±5.6 (95% CI: 30.5-52.5) months for the NTIT group (P=0.018). The 3-year survival rate was 12.5±11.5% for the conventional procedure group and 75.0±21.7% for the NTIT group (Figure 4).

DISCUSSION

A pancreaticoduodenectomy usually begins with kocherization, during which the draining vascular vessels from the tumor are preserved. This procedure has the potential of shedding cancer cells into the portal veins. Even though a curative operation is successfully performed, it is known that occult cancer cells exist in the patient's blood during and after surgery [9, 17, 18]. Hepatic metastasis is one of the major modes of pancreatic cancer recurrences [2, 3, 4, 19]. It has been suggested that the manipulation of tumors encourages viable cancer cells to shed into the blood stream and increases the incidence of hepatic metastases [18, 20]. Recently, many investigators focused on this problem and reported that the operation itself may cause cancer cells to disseminate into the blood stream in pancreatic surgery [14, 17]. Attention should also be paid to the dissemination of viable cancer cells into the lymphatic vessels, nerve bundles, and/or the peritoneal cavity. Postsurgical local recurrence and peritoneal dissemination are not rare in pancreatic cancer [2, 3, 4, 19]. In the NTIT group of this study, there was no peritoneal dissemination and a decreased hepatic recurrence rate. All lymphatic fluid samples squeezed from the resected cancerous pancreatic tissue were positive for CEA mRNA. These results suggest that there is a potential of such dissemination during the manipulation of tumors and this contributes to cancer recurrence. Early and frequent neural metastases can also be a cause of recurrent pancreatic cancer [21, 22, 23]. Consequently, at least some part of pancreatic cancer recurrence may be attributed to such intraoperative dissemination into portal venous and lymphatic vessels, and into the peritoneal cavity.

Pancreatic cancer cells produce a scatter factor-like activity which inhibits the intercellular bindings of cancer cells and augments their invasive ability [24, 25].

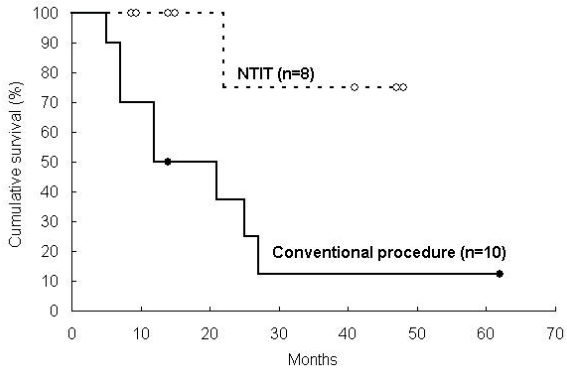

Figure 4. Cumulative survival (Kaplan-Meier) after resection of invasive pancreatic cancers. The 3-year survival rate was 14% for the conventional procedure group and 75% for the no-touch isolation group. Open and closed circles represent the survival periods of living patients in the NTIT group (n=7) and in the conventional procedure group (n=2), respectively.

Furthermore, pancreatic cancers often express CA19-9 on their cell surface [26]. CA19-9 is the ligand for E-selectin, which, during surgery, is expressed *de novo* on the vascular endothelial cells by cytokine action. Therefore, compared with other cancers, pancreatic cancer cells may be more susceptible to exfoliation from the main tumor and implantation in other organs. Once cancer cells enter into the blood stream, the implantation of cancer cells would be promoted, especially in the liver, the first filtering organ of portal blood. The NTIT has the potential of preventing the shedding of cancer cells through tumor manipulation. Kobayashi *et al*. also reported the use and efficacy of this pancreatectomy technique for periampullary cancers in order to prevent hepatic metastases [14].

We emphasize that the shedding of cancer cells can occur into the portal vein and into the lymphatic vessels. During the operation, if the lymphatic vessels are divided and left open, the shedding of cancer cells into the abdominal cavity and into retroperitoneal tissue can easily occur. Although curative surgery has been performed on patients with non-serosa-invasive gastric cancer, some patients have died from peritoneal recurrence. One of the postulations for peritoneal dissemination in non-serosa-invasive gastric cancer is that lymph node dissection opens lymphatic channels, which spreads viable cancer cells [15, 27]. Dissected lymphatic and vascular vessels should be completely blocked by surgical ligation or coagulation to prevent the dissemination of cancer cells. EIPL was also reported to be effective for the removal of free viable cancer cells during gastric cancer surgery [15, 27]. Therefore, in addition to NTIT, ligation of all lymphatic vessels and EIPL should be performed to improve the prognosis of resectable pancreatic cancer patients.

The results of this study suggest that some recurrences may be attributable to the intraoperative manipulation of the tumors. However, because the number of the patients enrolled in this study was small, further comparative study is necessary to confirm the significance of the NTIT procedure for pancreatic cancer surgery.

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Keywords Pancreatectomy; Pancreatic Neoplasms; Pancreaticoduodenectomy

Abbreviations DP: distal pancreatectomy; EIPL: extensive intraoperative peritoneal lavage; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; NTIT: no-touch isolation technique; SSPPD: subtotal stomach-preserving pancreaticoduodenectomy; UICC: International Union Against Cancer

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