portal or superior mesenteric vein invasion allowing for safe resection and reconstruction or T4 with tumor abutment of celiac or superior mesenteric artery not exceeded >180° of the circumference of the vessel wall on pre-treatment computed tomographic (CT) imaging, age between 20 and 79 years, life expectancy >6 months and an Eastern Cooperative Oncology Group performance status of 0 or 1. Pre-treatment evaluation included medical history, physical examination, chest radiograph, blood analysis (hemoglobin >9.0 g/dl, leukocyte count 4000–12 000 cells/ml, neutrophil >2000 cells/ml, platelets >100 000/ml, creatinine <1.2 mg/dl, total bilirubin <2.4 g/dl, serum aspartate aminotransferase level <2.5 times the upper normal limit (UNL) and serum alanine aminotransferase level <2.5 times UNL). After chemoradiotherapy, patients were reevaluated by CT imaging to assess resectability and response to the treatment. If deemed resectable for cure, patients underwent surgical exploration; those in whom the disease progressed to unresectable received additional chemotherapy. The follow-up was based on physical examination, laboratory examination and CT or magnetic resonance imaging every 3 months after study inclusion for the first 3 years and every 6 months for two further years until death or end of study participation due to other reasons.

Endpoints

The primary endpoints were toxicity for Phase I study and efficacy including pathologic response rate of chemoradiation for Phase II. The secondary endpoint were feasibility and tolerability for Phase I study and survival rate, time to treatment failure, response rate, resection rate, recurrence rate and local control rate. The response to treatment was classified into complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). CR was defined as disappearance of all target lesions; PR as at least 30% decrease in the sum of diameters of target lesions and no new lesions; PD at least 20% increase in the sum of diameters of target lesions or appearance of new lesions; SD neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. Survival was measured from the date of enrollment to the time of death from any cause. Time to treatment failure was defined as time from enrollment to the first observation of disease progression, death due to any cause or early discontinuation of treatment. Resection rate included the proportion of patients who successfully underwent complete surgical resection with microscopically negative margins.

Treatment Regimen

Chemotherapy

Patients were assigned to four sequential dose escalating cohorts of weekly GEM combined with twice-a-day accelerated radiotherapy (Table 2). Patients received GEM intravenously over 30 min at Days 1 and 8 with 400 mg/m^2 in Level 1, 2 and 600 mg/m^2 in Level 3 or 800 mg/m^2 in Level 4 and Phase II. Treatment was delayed and/or GEM doses were reduced by 20% for any Grade 3–4 non-hematological toxicities or Grade 4 hematological toxicities.

Radiotherapy

Radiotherapy was applied 5 days a week for 2 or 3 weeks. Twice-a-day irradiation of the primary pancreatic cancer and regional metastatic lymph nodes with a >1 cm diameter on CT scans was performed (two daily fractions of 1.5 Gy with a minimal 6 h interval between fractions). Four fields were used (Fig. 1), each being treated at each session. The total dose was 30 Gy (20 fractions/14 days) in Phase I Level 1 or 36 Gy (24 fractions/16 days) in Phase I Levels 2, 3, 4 and Phase II (Table 2).

Toxicity

Throughout the phases of therapy, patients were evaluated at least weekly by physical examination and laboratory tests to monitor for toxicity. Toxicity was graded by the National

Table 2. Dose escalation and toxicity profile of Phase I study

Level	Chemotherapy gemcitabine (GEM) (mg/m^2)	Radiotherapy 1.5 Gy/ fraction × 2 fractions/day	*n*	Toxicity							
				Hematological				Non-hematological			
				G0/1	G2	G3	G4	G0	G1	G2	G3/4
1	400	30	3		2	1			2	1	
2	400	36	3			3			3		
3	600	36	3			3		1	2		
4	800	36	6		3	2	1	1	5		

Patients received GEM intravenously at Days 1 and 8.
Twice-a-day irradiation of 1.5 Gy/fraction was applied on Days 1–5, 8–12 and 15–16.

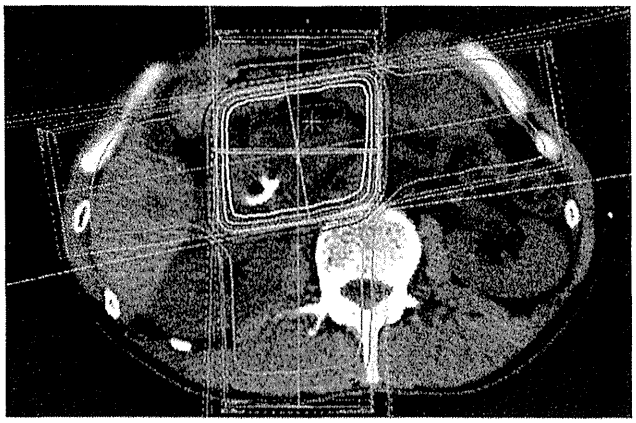


Figure 1. Irradiation field. The gross tumor volume represents the primary pancreatic tumor and regional metastatic lymph nodes.

Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 2.0 grading system. Treatment toxicity including gastrointestinal symptoms, fever, fatigue, leukopenia, neutropenia, thrombocytopenia, anemia, renal function and high liver function tests was monitored. Dose-limiting toxicity was defined as either the dose inducing Grade 4 neutropenia or Grade 3/4 thrombocytopenia or any Grade 3–4 non-hematological toxicity.

Statistical Considerations

The sample size was calculated from an expected pathologic response rate (Grade II or more) of 35% and a minimum response rate of 10% with an alpha error of 0.05 and beta error of 0.10. According to Simon's two-stage minimax design, 25 additional patients were required for step two. To minimize the sample size, the step-one patients with the RD were included into the step two. Disease-free survival and overall survival were computed according to the Kaplan–Meier method. The duration of survival was defined as time from enrollment. The endpoint represented any death for overall survival, and local recurrence or metastases for disease-free survival (including deaths observed during treatment). The median follow-up was 20.2 months (4.7–138.8) for the whole set of patients and 104.5 months (101.0–138.8) for the four living patients. All analyses were performed with StatView statistical software, version 5.0 (SAS Institute Inc.).

RESULTS

Patient Characteristics

Table 1 summarizes the patients' characteristics. Fifteen consecutive patients were enrolled in Phase I and 20 in Phase II (23 men, 12 women, median age, 71 years and range, 43–79). The median tumor size at presentation as measured by CT was 29.0 (range, 10.6–52.7) mm. Tumor staging performed before treatment was T3 in 20 patients and T4 in 15 patients. Five patients of the 35 had lymph node involvement.

Table 3. Overall patient toxicity profile

	Phase I (level 1–3) ($n = 9$)					Phase I (level 4) + Phase II ($n = 26$)				
	G0	G1	G2	G3	G4	G0	G1	G2	G3	G4
Hematological toxicities			2	7			2	9	13	2
Thrombocytopenia	4	3	2			7	13	2	3	1
Anemia		2	6	1		2	13	9	2	
Leucopenia			5	4			4	10	10	2
Neutropenia	1	3	1	4		5	5	7	8	1
Non-hematological toxicities	1	7	1			9	16	1		
Stomatitis	9					25	1			
Nausea	4	4	1			11	15			
Vomiting	6	2	1			21	5			
Diarrhea	9					20	5	1		
Skin	7	2				22	4			
Other	7	2	1			22	4			
Overall toxic effects			2	7			2	9	13	2

Treatment Delivery and Toxicity

Treatment compliance was excellent. A 100% of intended GEM for Day 1 and a mean of 84.4% of intended GEM for Day 8 were delivered in Phase I. Only one patient in Phase I interrupted the treatment without a radiation dose reduction. The Grade 3/4 toxicities of the NCI-CTC Version 2.0 grading system during chemoradiation included neutropenia 6/1, thrombocytopenia 1/0 and anemia 1/0, in Phase I. The dose-limiting toxicity was observed at Level 4 as a result of neutropenia in 1 of 6 patients (Table 2). After completion of the Phase I trial, irradiation with 36 Gy and GEM at 800 mg/m^2 were recommended for Phase II study. In Phase II study, the mean GEM dose was 800 mg/m^2 (100%) at first cycle and 710 mg/m^2 (88.8%) at second cycle. Eight patients interrupted their treatment without a radiation dose reduction. The Grade 3/4 toxicities during chemoradiation included neutropenia 6/0, thrombocytopenia 2/1 and anemia 2/0 in Phase II. No Grade 3 or 4 non-hematological toxicities were observed. Table 3 summarizes the maximum degree of acute toxicity during treatment. No major late toxicities were observed.

Surgical Procedure

In Phase I study, eight patients underwent standard pancreaticoduodenectomy (PD) and three underwent distal pancreatectomy with splenectomy (DP). Seven PD with portal vein reconstruction and one DP with en bloc celiac axis resection were performed. In Phase II study, 11 patients underwent PD and 4 underwent DP. Nine PD with portal vein reconstruction and two DP with partial portal vein resection were performed. The mean operative time for PD with the RD (Phase I Level 4

and Phase II) was 626 min (range, 485–1035 min), and the mean estimated blood loss in those patients was 1845 ml (range, 170–5500 ml). The mean operative time for DP with the RD was 282 min (range, 230–335 min), and the mean estimated blood loss in those patients was 548 ml (range, 170–920 ml). There were only three cases of Grade 1 and two cases of Grade 2 post-operative complications according to Clavien–Dindo's classification (15).

Overall Patient Response

Table 4 outlines the overall response to treatments. After the chemoradiotherapy, 6 patients of the 15 (40.0%) in Phase I study and 8 patients of the 20 (40.0%) in Phase II had a partial response. Twenty-six patients (6 patients in Level 4 of Phase I and 20 patients in Phase II) received the recommended dose. The response rate of patients who received the RD was 46.2% (12/26). Seven patients of the 15 (46.7%) in Phase I study and 10 patients of the 20 (50.0%) in Phase II had a SD. The disease of 3 (8.6%) of the 35 patients progressed to unresectable after chemoradiotherapy. One patient went off study after chemoradiotherapy as a result of superior mesenteric arterial aneurysm. Five patients were operated but not resected due to two cases of small liver metastasis and three cases of peritoneal dissemination. (Fig. 2). All unresected patients received GEM chemotherapy. No patient who progressed to unresectable responded to be resectable after additional chemotherapy. Eleven (73.3%) patients in Phase I study and 15 (75.0%) patients in Phase II patients underwent tumor resection after chemoradiotherapy with a complete R0 margin-negative resection. Any adjuvant chemotherapy was not performed.

Table 4. Tumor response to treatment

Phase	Level	*n*	Tumor response				Unresected	Evans grade				
			CR	PR	SD	PD		I	IIa	IIb	III	IV
I	1	3	0	0	3	0	2		1			
	2	3	0	1	1	1	1			1	1	
	3	3	0	1	2	0	1	1		1		
	4	6	0	4	1	1	0		2	4		
II		20	0	8	10	2	5		6	4	4	1
Total		35	0	14	17	4	9	1	9	10	5	1

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. See text for the definition of the response to therapy.

Of the 26 who underwent tumor resection, histopathological examination of the effects of chemoradiation was assessed using the grading system for the effect of chemoradiation reported by Evans et al. (16). None demonstrated a complete pathological response. In Phase I study, treatment effect was judged as Grade I in 1 patient, Grade II in 9 patients (Grade IIa in 3 and IIb in 6) and Grade III in 1 patient. In Phase II study, 10 patients were Grade II (Grade IIa in 6 and IIb in 4) and 5 were Grade III.

Patient Outcome and Survival

Twenty-six patients received complete R0 margin-negative resection. Four patients of the 15 (26.7%) in Phase I and 5 patients of the 20 (25.0%) in Phase II were considered to have unresectable tumors. Complete resection was performed in 21 patients of the 26 (80.8%) who received the recommended dose. (Fig. 2). Post-operatively, local recurrences

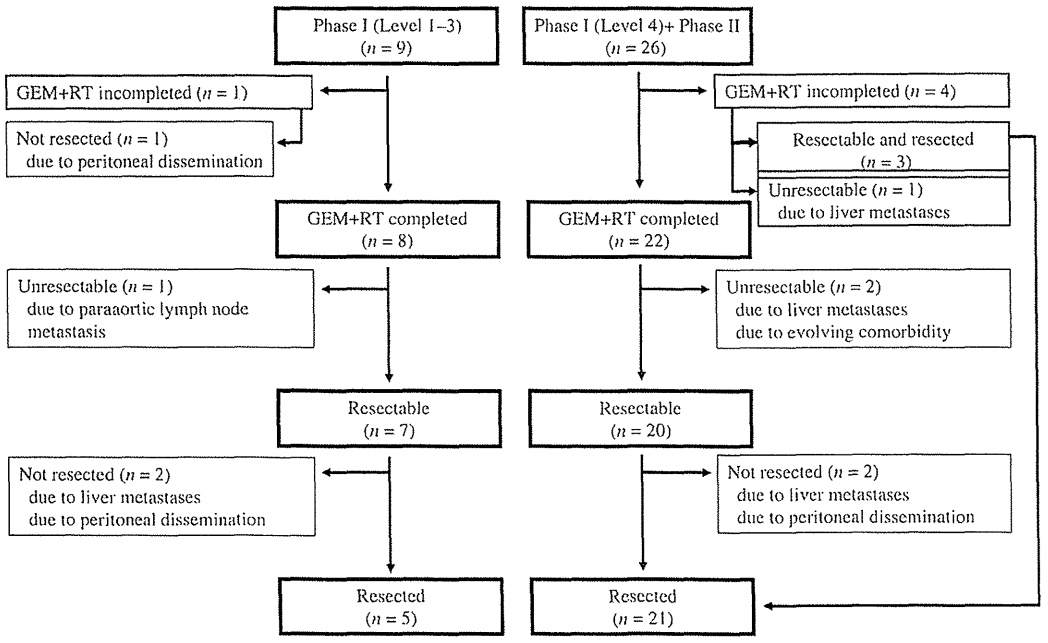


Figure 2. Flow diagram for the clinical processes of patients with borderline resectable pancreatic adenocarcinoma.

were noted in 3 patients of the 26 resected patients (11.5%) in Phase I and II. The local control rate in patients who received the RD was 85.7% (18/21). Liver metastasis occurred in 11 patients of the 26 resected patients (42.3%). The recurrence rate for patients who received the RD was 85.7% (18/21) (Table 5). Eighteen events were considered for disease-free survival analysis (Fig. 3). The median disease-free survival rate for patients who underwent resection followed by the RD was 17.4 months. The median survival time was 21.0 months for patients with the RD and 41.2 months for those who underwent tumor resection. Five patients survived >5 years. These long survivors received resection with treatment response of PR or SD. PR and SD were three and two cases, respectively.

DISCUSSION

Previous studies described the benefits of multimodal therapy compared with radiotherapy alone in patients with unresectable disease (17,18). For the multimodal therapy, pre-operative chemoradiation provides the following benefits (16): (i) tumors may be downstaged following pre-operative chemoradiation to achieve margin-negative resection. (ii) Radiation therapy is more effective when applied to well-oxygenated cells with intact vascularization. (iii) Pre-operative chemoradiation may identify those patients with occult distant metastasis or rapidly progressive disease on repeat staging studies after chemoradiation and thus avoid useless surgery (19,20). (iv) Pre-operative chemoradiation may also reduce cancer cell seeding during surgery and (v) Full courses of chemoradiation can be delivered pre-operatively without potential delays caused by surgical complications and prolonged recovery times, a frequent problem in adjuvant therapy studies.

Randomized trials in patients with advanced pancreatic cancer demonstrated the superior survival benefits of GEM compared with 5FU (12,21,22). GEM also improved cancer-related symptoms and performance status in both treatment-naive and previously treated patients with metastatic disease. In addition, laboratory studies have demonstrated that GEM also has potent radiosensitizing properties (23–27). Therefore, we decided to use a combination of GEM and radiotherapy. Accelerated hyperfractionation radiation shortens the overall treatment duration with same total radiotherapy equivalent dose. It also increases the local control rate without increasing gastrointestinal toxicity (28). Therefore, we selected accelerated hyperfractionation radiation combined with GEM for pre-operative therapy. The overall toxicity profile of this chemoradiation regimen was excellent, with no Grade 3 or 4 gastrointestinal toxicities.

Wolff et al. (29) reported Phase I trial of GEM combined with radiation in patients with locally advanced pancreatic cancer. They suggested a mean GEM therapeutic dose of 350 mg/m^2/week for 7 weeks, when GEM is given weekly with concomitant radiotherapy at a dose of 30 Gy in 10 fractions. After completion of the Phase I trial, the M D. Anderson Cancer Center group recommended a higher GEM dose of 400 mg/m^2/week for Phase II investigation. On the other hand, considering the clinical benefits associated with the use of GEM as a systemic agent, a standard dose of GEM (1000 mg/m^2/week) was used and the tolerable radiation dose that could be delivered to the primary tumor was investigated by McGinn et al. (26). After completion of Phase I trial, 36 Gy in 2.4 Gy fractions was recommended for Phase II investigation with a standard dose of GEM (1000 mg/m^2/week) (30). Our median survival time of patients who received the RD and resected was 41.2 months. It was better than 34 months of reduced GEM dose of 400 mg/m^2/week with 30 Gy or 26 months of full GEM dose of 1000 mg/m^2/week with 36 Gy.

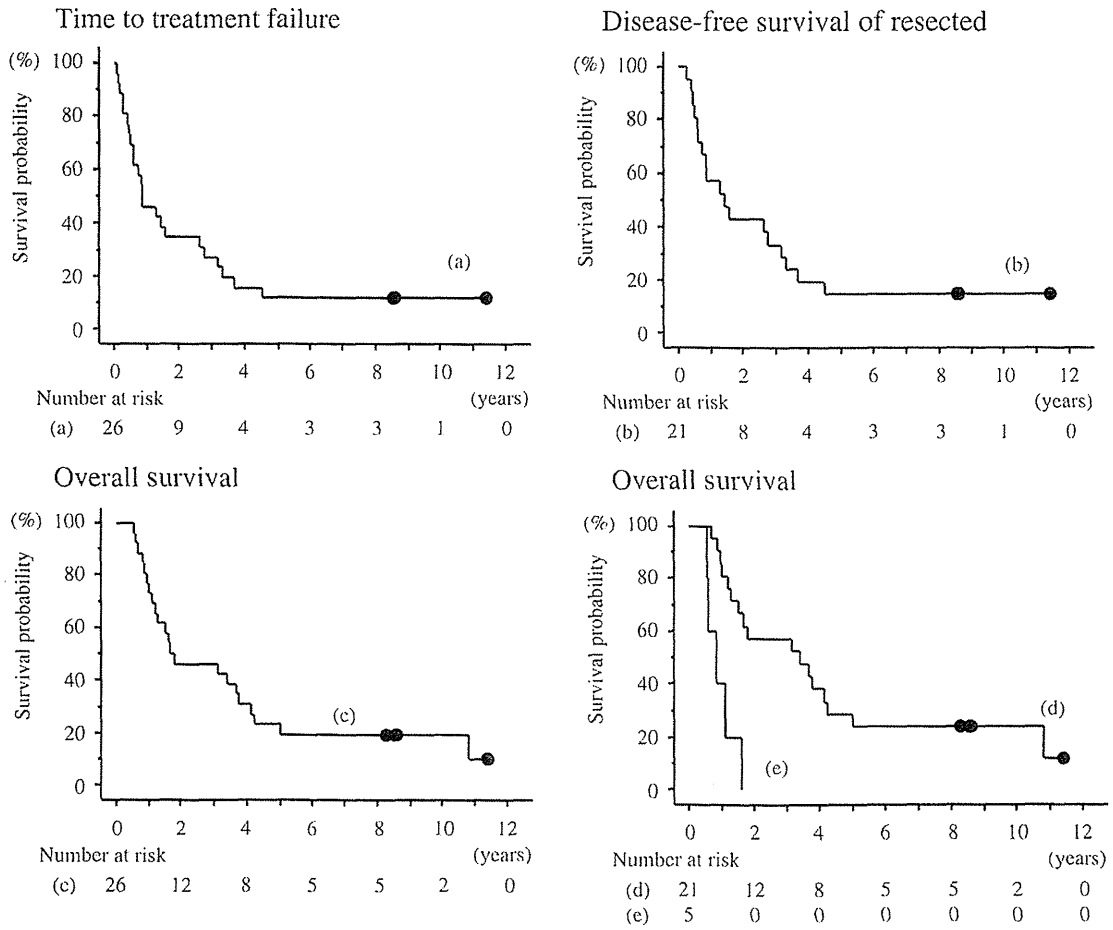
One theoretical disadvantage of chemoradiation before surgery is toxicity and radiation-related changes within the tissues in the surgical field that may increase the risk of operation for morbidity and mortality (19). Project study by the Japanese Society of Hepato-Biliary-Pancreatic Surgery described that operation time was significantly longer in the neoadjuvant group (567 min, range 190–1160) than in the surgery-first group (496 min, range 161–1221) ($P = 0.0005$), but there were no between group differences in blood loss (1400 ml range 60–8422 vs. 1137 ml range 20–16201) ($P = 0.16$), respectively (31). Our mean operative time and blood loss for PD and DP with the RD was 626 min (range, 485–1035 min) and 282 min (range, 230–335 min), 1845 ml (range, 170–5500 ml) and 548 ml (range, 170–920 ml), respectively. There were no surgery-related complications that could be attributed to the pre-operative therapy.

A pathological CR in the resected pancreas after pre-operative chemoradiation is rare in patients with adenocarcinoma of the pancreas (19). Evans et al. (16) reported that Grade IIb or more extensive destruction of tumor (>50%) was seen in 7 of the 17 (41%) patients. Our data on histopathological assessment of the effect of treatment indicated that 13 of the 21 (61.9%) patients who received the RD followed by resection had Grade IIb or more chemoradiation treatment effect (>50% of tumor cells were destroyed) according to the grading system of Evans et al. (15). The reported rate of Grade II or more chemoradiation treatment effect for 5FU,

Table 5. Recurrence site after surgical resection

Phase	Level	*n*	Resected	Recurrence				
				Local	Liver	PER	PUL	Total
I	1	3	1	0	0	1	0	1
	2	3	2	0	2	0	0	2
	3	3	2	0	1	0	1	2
	4	6	6	1	3	0	1	5
II		20	15	2	5	3	3	13
Total		35	26	3	11	4	5	23

PER, peritoneal; PUL, lung.



Survival	n	1 year (%)	5 years (%)	Median (months)	
Time to treatment failure	26	46.2	11.5	10.6	(a)
Disease-Free Survival of Resected	21	57.1	14.3	17.4	(b)
Overall Survival	26	76.9	23.1	21.0	(c)
Overall Survival of Resected	21	85.7	28.6	41.2	(d)
Overall Survival of Unresected	5	40.0	0.0	10.0	(e)

Figure 3. Time to treatment failure, disease-free survival and overall survival of patients who received the recommended dose.

paclitaxel, GEM is 20.0–41.2, 21 and 23.5–67.7%, respectively (19,20,30,32). Considered together, we speculate that GEM does not only have a superior systemic therapeutic effect but also has radiosensitizing properties greater than those of 5FU and paclitaxel.

A meta-analysis of pre-operative therapy in pancreatic adenocarcinoma was reported (33–35). In patients with initially resectable tumors, resection frequencies and survival after neoadjuvant therapy are similar to those of patients with primarily resected tumors and adjuvant therapy. Neoadjuvant treatment appears to have some activity in patients with borderline or unresectable pancreatic adenocarcinoma. Ten neoadjuvant studies with 182 participants with borderline resectable cancer were analyzed by a meta-analytical approach (36). The 107 patients were resected and the proportion of R0 resection amounted to 83%. The result of the meta-analysis of pre-operative therapy for patients with localized pancreatic cancer indicates a potential advantage for a minority of those with borderline or unresectable lesions. The meta-analysis of neoadjuvant chemoradiotherapy for patients with borderline resectable pancreatic cancer indicated the weighted mean of median survival amounted to 12.4 months (range, 9–16 months) for the overall cohort of patients, 22.0 months (range, 12–32 months) for those who were resected and 9.7 months

(range, 8–41 months) for unresected patients (36). Our overall median survival of 21.0 months in patients who received the RD and 41.2 months in those who underwent resection indicates the potential of pre-operative therapy to improve the outcome of patients with borderline resectable pancreatic cancer. Three of the 21 (14.3%) patients who underwent surgical resection remain alive without evidence of disease recurrence at a minimum follow-up of 104 months.

In conclusion, the present study demonstrated that pre-operative GEM-based chemoradiotherapy is well tolerated and safe. Our protocol allowed a high rate of subsequent resection, with encouraging survival data. Multimodal approaches using pre-operative GEM-based chemoradiotherapy followed by surgery and adjuvant chemotherapy would be a promising protocol for borderline resectable pancreatic cancer.

Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

Risk factors for 6-month continuation of S-1 adjuvant chemotherapy for resected pancreatic cancer

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Abstract
Background The factors which affect the 6-month continuation of adjuvant chemotherapy with S-1 have not been fully evaluated in pancreatic cancer. The objective of this retrospective study was to clarify the risk factors for the discontinuation of S-1 adjuvant chemotherapy after 6 months of treatment.
Methods The study included patients who underwent curative surgery for pancreatic cancer, were diagnosed with stage II or III disease, had a serum creatinine level ≤1.2 mg/dl and received adjuvant S-1 between June 2007 and March 2014.
Results Forty patients were eligible for the present study. A comparison of the 6-month continuation stratified by each clinical factor using the log-rank test revealed a significant difference in the creatinine clearance (CCr) between the patients who continued and discontinued the treatment. A CCr of 60 ml/min was regarded as a critical point. The uni- and multivariate Cox's proportional hazard analyses demonstrated that the CCr was the only significant independent predictive factor. The 6-month continuation rate was 70.8 % in the patients with a CCr ≥60 ml/min and was 25.0 % in patients with a CCr <60 ml/min ($P = 0.008$). The patients with a CCr <60 ml/min developed adverse events more frequently and earlier than those with a CCr ≥60 ml/min.
Conclusions A CCr < 60 ml/min was a significant risk factor for the 6-month discontinuation of S-1 adjuvant chemotherapy in pancreatic cancer patients, even though the renal function was judged to be normal based on the serum creatinine level. Careful attention is therefore required to improve the S-1 continuation in patients with a CCr < 60 ml/min.

Keywords Pancreatic cancer · Adjuvant chemotherapy · S-1 · Continuation · Renal function

Introduction

Pancreatic cancer is one of the major causes of cancer death worldwide, with a 5-year survival rate of <5 % [1, 2]. In 2013, the Japan Adjuvant Study Group of Pancreatic Cancer (JASPAC-01) phase III trial demonstrated that S-1 is effective as adjuvant chemotherapy for Japanese patients who have underwent curative surgery for pancreatic cancer and were diagnosed with pathological stage II or III disease [3]. Therefore, adjuvant chemotherapy with S-1 after curative surgery is considered the standard therapy for these patients in Japan.

The aim of adjuvant chemotherapy was to eradicate micrometastatic tumor cells. As a result, it is essential to continue chemotherapy for a minimal length of time to ensure that these cells are eradicated. Six months of

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treatment are considered to be necessary for pancreatic cancer based on the JASPAC-01. The efficacy of adjuvant chemotherapy decreased when the treatment was insufficient in breast cancer patients [4].

Although adjuvant chemotherapy with S-1 after curative surgery is considered the standard therapy for these patients in Japan, the continuation of S-1 adjuvant chemotherapy is relatively low. Indeed, Fukutomi et al. reported that the proportion of patients with treatment failure was 72 % at 6 months after surgery, and the most common cause of withdrawal was the occurrence of adverse events.

To improve the patient survival, it is important to identify risk factors for the continuation of S-1, because physicians may need to provide better support for the patients who have such risk factors. This study investigated the risk factors which affect the 6-month continuation of adjuvant chemotherapy with S-1 for pancreatic cancer patients who underwent curative surgery.

Patients and methods

Patients

The patients were selected from the retrospective database of the Kanagawa Cancer Center, Department of Gastrointestinal Surgery, Yokohama, Japan, according to the following criteria: (1) patients had histologically proven pancreatic adenocarcinoma, (2) patients underwent a curative surgery for pancreatic cancer as a primary treatment between June 2007 and March 2014, (3) patients had stage IIA, IIB or III disease diagnosed pathologically according to the 6th edition of the UICC [5], (4) patients started adjuvant chemotherapy with S-1 at a dose of 80 mg/m^2 within 10 weeks after surgery and (5) the serum creatinine level was less than the upper limit of the normal range ($\leq$1.2 mg/dl).

Treatment

The patients received S-1 chemotherapy and were followed on an out-patient basis. Two groups of patients received S-1: (1) as a test arm of the JASPAC-01 trial and (2) as part of general clinical practice. The patients who were registered in the JASPAC-01 trial received 40 mg of S-1 per square meter of body surface area twice a day for 4 weeks, followed by 2 weeks of rest as one course (6-week schedule), and this was continued for 6 months after surgery. The remaining patients received S-1 at the same dose as part of routine clinical practice for 6 months following the protocol of the JASPAC-01 after the results of the JASPAC-01 were reported. The patients with a body surface area <1.25 m^2 received 80 mg daily, those with a body surface area of 1.25 m^2 or more, but <1.5 m^2, received 100 mg daily and those with a body surface area of 1.5 m^2 or more received 120 mg daily.

The need for a reduction in the starting dose, a suspension or delay in treatment or a dose reduction was determined by the protocol for the clinical trial in the patients registered in the JASPAC-01 trial [6]. The need for a reduction of the starting dose, a delay in treatment or a dose reduction in the patients who received S-1 in clinical practice was determined following the findings of the JASPAC-01 trial. Briefly, the treatment was delayed when patients had hematological adverse events of grade 3 or more, or non-hematologic adverse events of grade 2 or more, until all adverse events recovered to grade 0 or 1, and then was started at a reduced dose of 100, 80 or 50 mg, based on the body surface area described above. The patients who started with the 6-week schedule of S-1 and experienced the adverse events described above at a reduced dose were switched from the 6-week schedule to a 3-week schedule (2 weeks of treatment followed by 1 week of rest).

Follow-up during S-1 treatment

In principal, the patients who received S-1 underwent hematologic tests and assessments of clinical symptoms every 2 weeks. The presence of a relapse was determined by means of imaging studies, including ultrasonography, computed tomography (CT), gastrointestinal radiography series and endoscopy. Patients underwent at least one type of imaging study, usually CT, at 3-month intervals during S-1 treatment.

Evaluation and statistical analyses

The toxicities were graded according to the National Cancer Institute common toxicity criteria, version 3.0. Renal impairment was measured in terms of the CCr, calculated by the formula proposed by Cockroft and Gault [7]. The time to S-1 treatment failure (TTF), and the proportions of treatment failures at 3 and 6 months after surgery were calculated using the Kaplan–Meier method and were compared by the log-rank test. In this study, when S-1 was discontinued <6 months after surgery, we defined that the event occurred on the day when the last date of S-1 treatment based on the study protocol, the patient's refusal due to the adverse events, the patient's refusal due to other reasons than the adverse events, due to disease recurrence or due to the patient's death. Cox's proportional hazard model was used to perform uni- and the stepwise multivariate analyses to determine the risk factors for the continuation of S-1 chemotherapy. A P value <0.05 was considered to be statistically significant. The SPSS software package (v11.0 J Win, SPSS, Chicago, IL, USA) was used for all statistical analyses.

Table 1 Comparison of continuation by patient characteristics

Characteristics	No. of patients (%)	3-months continue rate (%)	6-months continue rate (%)	*P* value
Age (years)				0.495
– < 75	32 (80)	66.3	62.7	
75 ≤ –	8 (20)	52.5	52.5	
Gender				0.696
Male	24 (60)	65.2	60.6	
Female	16 (40)	65.2	65.2	
Type of surgery				0.780
DP	11 (27.5)	72.7	63.6	
PD or TP	29 (72.5)	59.3	59.3	
Surgical complication				0.975
Yes	9 (22.5)	57.1	57.1	
No	31 (77.5)	65.2	61.2	
Body weight loss (%)				0.923
– < 10 %	27 (67.5)	65.3	60.6	
10 % ≤ – < 15 %	7 (17.5)	57.1	57.1	
15 % ≤ –	6 (15)	66.7	66.7	
Creatinine clearance (ml/min)				<0.001
– < 60	7 (17.5)	14.3	14.3	
60 ≤ – < 70	9 (22.5)	87.5	87.5	
70 ≤ –	24 (60)	71.3	65.8	
Stage				0.310
IIA	8 (32.5)	73.9	73.9	
IIB	32 (67.5)	59.3	55.1	

DP distal pancreatomy, *PD* pancreaticoduodenectomy, *TP* total pancreatomy

Results

A total of 112 patients underwent surgical resection and were pathologically diagnosed with stages IIA, IIB or III disease. Forty patients were finally eligible for the present study. Eighteen patients had been registered to the JASPAC-01 trial between June 2007 and June 2010. Twenty-two received S-1 treatment in clinical practice after the results of the JASPAC-01 were reported. The patients' ages ranged from 47 to 81 years (median 68 years). Twenty-four patients were male and 16 were female. The median serum creatinine level before adjuvant treatment was 0.66 mg/dl (range 0.39–1.07 mg/dl), and the median creatinine clearance (CCr) before adjuvant treatment was 70.3 ml/min (range 33.7–125.3 ml/min).

A comparison of the 6-month continuation stratified by each clinical factor by the log-rank test revealed that there was a significant difference in the CCr between the patients who continued and discontinued treatment (Table 1). A CCr of 60 ml/min was defined as the optimal critical point of classification considering the 3- and 6-month continuation rates. Each clinicopathological factor was categorized as shown in Table 2 and was analyzed for the risk of S-1 discontinuation. Univariate analyses demonstrated that the CCr was the only significant risk factor for discontinuation. The CCr was therefore selected for the final model to be analyzed by the multivariate analysis (Table 2). Figure 1 shows that the rate of treatment failure at 6 months was 70.8 % in patients with a high CCr and was 25.0 % in those with a low CCr. Six patients stopped S-1 within 6 months after surgery due to recurrence.

Table 3 shows the details of the patients who stopped S-1 due to any events. The reasons for discontinuation included the pre-specified rules of the protocol for the development of adverse events in five patients, the patient's refusal due to other reasons than the adverse events in two patients, disease recurrence in six patients, but death did not occur in any of the patients. The results showed that adverse events occurred more frequently and earlier in the patients with a low CCr than in those with a high CCr. About half of the patients with a low CCr could not continue S-1 for more than 1 month. Although these patients were informed of the possibility that continuation might be possible through dose attenuation or changing the treatment schedule, all refused further S-1 adjuvant treatment.

The CCr remained a significant factor in both the univariate and multivariate analyses in the subset excluding the six patients who discontinued S-1 due to recurrence

Table 2 Uni and multivariate cox proportional hazards analysis of clinicopathological factors

Factors	Number	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Age (years)				0.503			
– < 75	32	1.000					
75 ≤ –	8	1.551	0.430–5.603				
Gender				0.699			
Female	16	1.000					
Male	24	1.243	0.413–3.736				
Type of surgery				0.782			
DP	11	1.000					
PD or TP	29	1.179	0.368–3.780				
Surgical complication				0.975			
No	31	1.000					
Yes	9	1.021	0.285–3.661				
Body weight loss (%)				0.871			
– < 10 %	27	1.000					
10 % ≤ –	13	1.095	0.366-3.269				
Creatinine clearance (ml/min)				0.008			0.008
60 ≤ –	33	1.000					
– < 60	7	4.202	1.443–12.237		4.202	1.443–12.237	
Stage				0.322			
IIA	8	1.000					
IIB	32	1.906	0.531–6.841				

DP distal pancreatomy, *PD* pancreaticoduodenectomy, *TP* total pancreatomy

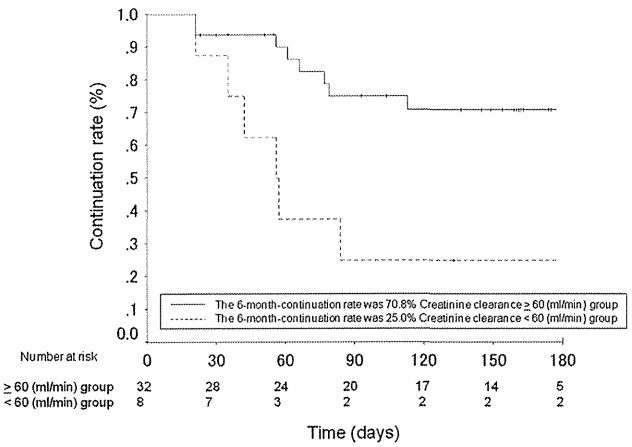

Fig. 1 A comparison of the 6-month continuation rates between the patients who had a creatinine clearance >60 ml/min and those who had a creatinine clearance <60 ml/min

(Table 4). In this subset, the rate of treatment failure at 6 months was 84.2 % in the patients with a high CCr and was 26.7 % in those with a low CCr (Fig. 2).

Discussion

This report demonstrated that a CCr < 60 ml/min was a significant risk factor for the discontinuation of S-1 adjuvant chemotherapy in pancreatic cancer patients, even though the renal function was judged to be normal based on the serum creatinine level. Therefore, it is necessary to calculate the CCr level when starting S-1 adjuvant chemotherapy. Careful attention is required for S-1 continuation in patients with a CCr < 60 ml/min. Moreover, only about one-fourth of the patients with a CCr < 60 ml/min could continue S-1 for 6 months, suggesting that these patients had little chance to benefit from S-1 adjuvant chemotherapy.

S-1 is an oral fluoropyrimidine containing tegafur (a prodrug of fluorouracil), 5-chloro-2, 4-dihydropyrimidine (CDHP) and potassium oxonate. CDHP is an inhibitor of dihydropyrimidine dehydrogenase (DPD), which is the rate-limiting enzyme for the degradation of fluorouracil [8]. The clearance of CDHP is reduced by renal dysfunction, resulting in a high blood concentration of 5-FU due to decreased DPD activity [9, 10]. Similar results were observed in gastric cancer studies. We previously demonstrated that a CCr < 60 ml/min was a significant risk factor for the discontinuation of S-1 adjuvant chemotherapy, even though the renal function was judged to be normal by the serum creatinine level. Moreover, only half of the patients with a CCr < 60 ml/min could continue S-1 for more than 1 month [11]. In addition, Yamanaka et al. reported that baseline renal impairment is a significant risk factor for grade 3–4 adverse events caused by S-1 chemotherapy in patients with advanced gastric cancer. They showed that the incidence of key severe adverse events,

Table 3 The details of the withdrawal between creatinine clearance ≥60 (ml/min) group and Creatinine clearance <60 (ml/min) group

Case no.	Creatinine clearance ≥60 (ml/min) group			Case no.	Creatinine clearance <60 (ml/min) group		
	The reasons for withdrawal				The reasons for withdrawal		
	Day	Type	Grade		Day	Type	Grade
1	21	Recurrence	–	1	21	Diarrhea	2
2	21	Patients' refuse	–	2	42	Fatigue	2
3	56	Recurrence	–	3	56	Recurrence	–
4	61	Recurrence	–	4	57	Cholangitis	3
5	66	Diarrhea	2	5	84	Recurrence	–
6	77	Recurrence	–				
7	79	Patients' refuse	–				
8	113	Gastric ulcer	3				

Table 4 Uni and multivariate cox proportional hazards analysis of clinicopathological factors in the subset excluding eight patients who discontinue S-1 because of recurrence

Factors	Number	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Age (years)				0.460			
– < 75	27	1.000					
75 ≤ –	7	1.836	0.366–9.197				
Gender				0.814			
Female	14	1.000					
Male	20	1.188	0.282–5.014				
Type of surgery				0.712			
DP	9	1.000					
PD or TP	25	1.354	0.271–6.755				
Surgical complication				0.658			
No	27	1.000					
Yes	7	1.605	0.197–13.062				
Body weight loss (%)				0.810			
– < 10 %	23	1.000					
10 % ≤ –	11	1.192	0.285–4.995				
Creatinine clearance (ml/min)				0.007			0.007
60 ≤ –	28	1.000					
– < 60	6	6.870	1.685–28.004		6.870	1.685–28.004	
Stage				0.981			
IIA	8	1.000					
IIB	26	1.017	0.242–4.271				

DP distal pancreatomy, *PD* pancreaticoduodenectomy, *TP* total pancreatomy

such as neutropenia, nausea/vomiting, diarrhea, stomatitis, rash and skin pigmentation, were significantly higher in the patients with a CCr level under 50 ml/min than in those who had a CCr level over 80 ml/min [12]. Indeed, three of seven patients with a low CCr in the current series stopped S-1 adjuvant chemotherapy, whereas only two of 33 patients with a high CCr discontinued treatment due to toxicities. Moreover, all patients with a low CCr stopped S-1 adjuvant chemotherapy in the first 2 months due to toxicities, whereas no patients with a high CCr withdrew from therapy by that time. Adverse events occurred more frequently and earlier in the patients with a low CCr than in those with a high CCr.

A comparison of the present findings with the results of the JASPAC-01 trial revealed that the continuation rate tended to be lower in the present study. The clinical trial had strictly defined rules for the discontinuation of S-1, while the daily clinical practice cohort did not. In the daily clinical practice cohort, even though the physicians explained the details of the JASPAC-01 trial to the patients,

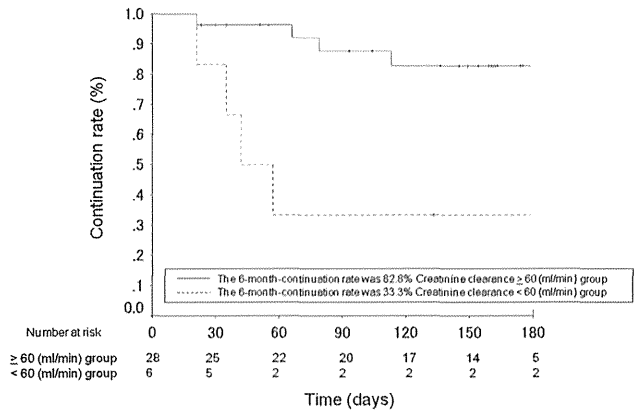

Fig. 2 A comparison of the 6-month continuation rates between the patients who had a creatinine clearance >60 ml/min and those who had a creatinine clearance <60 ml/min in the subset that excluded six patients who discontinued S-1 treatment because of recurrence

S-1 was sometimes discontinued due to grade 1–2 non-hematological toxicities such as anorexia, fatigue and nausea, because the quality of life of the patients was affected more by non-hematological toxicity than by hematological toxicity. These factors might have affected the present results.

Special attention is required when interpreting the current results because there were several potential limitations associated with this study. First, the optimal cutoff value for the CCr is unclear. When comparing the 3-month continuation rate and the 6-month continuation rate based on the CCr using the log-rank test, both continuation rates were clearly separated by a CCr of 60 ml/min. However, the cutoff value might depend on the patient's backgrounds. Thus, an appropriate cutoff value should be determined in other validation studies in other populations. Second, there might be some selection bias in the present study. Generally, the patients treated at specialized cancer center usually have relatively good condition and less serious co-morbidities than those treated at most Japanese general hospital. Therefore, it is unclear that our results have the generalizability. Third, this study was a retrospective single center study with a small sample size. Our findings might be by chance. In this study, we calculated the total days of S-1 treatment based on the patient's record. Because the data were based on the patients' records, it is difficult to know whether the patients actually took the medication. Thus, the TTF might not be accurate. Considering these limitations, the current results should be validated in other series with a larger number of patients.

In summary, a CCr < 60 ml/min was a significant risk factor for the discontinuation of S-1 adjuvant chemotherapy, even though the renal function was judged to be normal based on the serum creatinine level. Careful attention is required to improve the S-1 continuation in patients with a CCr < 60 ml/min.

Conflict of interest None.

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CASE CONTROL STUDY

Case-control study of diabetes-related genetic variants and pancreatic cancer risk in Japan

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Abstract

AIM: To examine whether diabetes-related genetic variants are associated with pancreatic cancer risk.

METHODS: We genotyped 7 single-nucleotide polymorphisms (SNPs) in *PPARG2* (rs1801282), *ADIPOQ* (rs1501299), *ADRB3* (rs4994), *KCNQ*1 (rs2237895), *KCNJ11* (rs5219), *TCF7L2* (rs7903146), and *CDKAL1* (rs2206734), and examined their associations with pancreatic cancer risk in a multi-institute case-control study including 360 cases and 400 controls in Japan. A self-administered questionnaire was used to collect detailed information on lifestyle factors. Genotyping was performed using Fluidigm SNPtype assays. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between these diabetes-associated variants and pancreatic cancer risk.

RESULTS: With the exception of rs1501299 in the

ADIPOQ gene (P = 0.09), no apparent differences in genotype frequencies were observed between cases and controls. Rs1501299 in the *ADPIOQ* gene was positively associated with pancreatic cancer risk; compared with individuals with the AA genotype, the age- and sex-adjusted OR was 1.79 (95%CI: 0.98-3.25) among those with the AC genotype and 1.86 (95%CI: 1.03-3.38) among those with the CC genotype. The ORs remained similar after additional adjustment for body mass index and cigarette smoking. In contrast, rs2237895 in the *KCNQ1* gene was inversely related to pancreatic cancer risk, with a multivariable-adjusted OR of 0.62 (0.37-1.04) among individuals with the CC genotype compared with the AA genotype. No significant associations were noted for other 5 SNPs.

CONCLUSION: Our case-control study indicates that rs1501299 in the ADIPOQ gene may be associated with pancreatic cancer risk. These findings should be replicated in additional studies.

Key words: Single-nucleotide polymorphisms; Pancreatic cancer; Risk; Case-control study; Odds ratio

Core tip: Although it is likely that a common genetic background predisposes individuals to developing both diabetes and pancreatic cancer, very few molecular epidemiologic studies have addressed this issue. We therefore genotyped 7 diabetes-related genetic variants and found that rs1501299 in the ADIPOQ gene may be associated with pancreatic cancer risk. The role of adiponectin variants needs further study.

Kuruma S, Egawa N, Kurata M, Honda G, Kamisawa T, Ueda J, Ishii H, Ueno M, Nakao H, Mori M, Matsuo K, Hosono S, Ohkawa S, Wakai K, Nakamura K, Tamakoshi A, Nojima M, Takahashi M, Shimada K, Nishiyama T, Kikuchi S, Lin Y. Case-control study of diabetes-related genetic variants and pancreatic cancer risk in Japan. *World J Gastroenterol* 2014; 20(46): 17456-17462 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i46/17456.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i46.17456

INTRODUCTION

The etiology of sporadic pancreatic cancer remains largely unknown. Epidemiologic studies have consistently shown that pancreatic cancer is positively associated with cigarette smoking and long-standing diabetes[1,2]. A 2005 meta-analysis reported that the risk for pancreatic cancer is 82% higher among diabetics compared with those without diabetes[3], though it is unclear which factors underlying diabetes are associated with pancreatic cancer. Most epidemiological studies have been limited by self-reporting of diabetes and by the lack of objective biomarkers, such as fasting plasma glucose or insulin levels, to address the temporal relationship between diabetes and pancreatic cancer. There is increasing evidence from clinical studies that pancreatic cancer induces new-onset diabetes[4,5]. The evidence available thus far strongly suggests that the relationship between diabetes and pancreatic cancer is bi-directional.

Given the well-recognized, positive association between type 2 diabetes and pancreatic cancer risk in epidemiological studies, it may be interesting to examine whether diabetes-related genetic variants may also be associated with pancreatic cancer risk. Genome-wide association studies (GWAS) have reported that at least 30 loci are associated with susceptibility to diabetes in various populations, with the majority originating from individuals of European descent[6]. Because of the potential differences in fat distribution and genetic background between Asian and Western populations[7,8], we focused on diabetes-related genetic variants reported in studies of Japanese populations, and variants that were first reported in GWAS of other populations and then replicated in Japanese populations. Among the 7 diabetes susceptibility genes we chose for the present study, *PPARG2*, *ADIPOQ*, and *ADRB3* have been shown to be closely associated with diabetes risk in Japanese subjects[9]; *KCNQ1* was reported as a diabetes susceptibility gene simultaneously by 2 independent Japanese research groups in 2008[10,11]; *KCN11*, *TCF7L2*, and *CDKAL1* were also reported to be associated with diabetes susceptibility in GWAS of Japanese subjects[12,13].

Although it is likely that a common genetic background predisposes individuals to developing both diabetes and pancreatic cancer, very few molecular epidemiologic studies have addressed this issue. We hypothesized that diabetes susceptibility genetic variants may be associated with an increased risk of pancreatic cancer in Japanese subjects. We therefore genotyped 7 single-nucleotide polymorphisms (SNPs) in *PPARG2* (rs1801282), *ADIPOQ* (rs1501299), *ADRB3* (rs4994), *KCNQ1* (rs2237895), *KCNJ11* (rs5219), *TCF7L2* (rs7903146), and *CDKAL1* (rs2206734) and examined their associations with pancreatic cancer risk in a multi-institute, case-control study in Japan.

MATERIALS AND METHODS

Study subjects

The purpose of our case-control study was to evaluate the role of genetic polymorphisms and gene-environment interactions in the development of pancreatic cancer in Japanese subjects. The details of the study design have been described elsewhere[14]. Briefly, cases were defined as patients who were newly diagnosed with pancreatic ductal adenocarcinoma at five participating hospitals from April 1, 2010, through May 15, 2012. A diagnosis was made according to imaging modalities and further confirmed by pathology reports. Pathologically confirmed cases represented approximately 90% of all cases in this study. During the same time period, we recruited the majority of control subjects from in-

Table 2 Selected characteristics of cases and controls *n* (%)

	Cases *n* = 360	Controls *n* = 400
Age, mean ± SD	67.8 ± 8.8	64.8 ± 9.5
Sex		
Male	215 (59.7)	226 (56.5)
Female	145 (40.3)	174 (43.5)
BMI, mean ± SD	22.9 ± 3.3	22.8 ± 3.2
History of diabetes		
Yes	87 (24.1)	35 (8.7)
No	269 (74.7)	362 (90.5)
Cigarette smoking		
Ever	215 (59.7)	198 (49.5)
Never	145 (40.2)	202 (50.5)
Age upon starting smoking (mean ± SD)	21.8 ± 4.8	20.5 ± 4.5
Number of cigarettes smoked per day (mean ± SD)	20.3 ± 9.0	16.2 ± 9.2

BMI: Body mass index.

Table 2 Single-nucleotide polymorphisms profile

Rs number	Gene	Chromosome location	Risk allele[1]	Alternative allele
rs1801282	*PPARG2*	3p25	C	G
rs1501299	*ADIPOQ*	3q27	C	A
rs4994	*ADRB3*	8p12	C	T
rs2237895	*KCNQ1*	11p15	C	A
rs5219	*KCNJ11*	11q23	T	C
rs7903146	*TCF7L2*	10q25	T	C
rs2206734	*CDKAL1*	6p22	A	G

[1]Based on the odds ratios reported for the association between T2D risk allele and T2D risk in previous studies.

patients and outpatients as well as from individuals who underwent medical checkups at one of the participating hospitals. None of the control subjects had a history of cancer. The diagnoses for hospital control subjects included a variety of diseases, such as anemia, gastric ulcers, and irritable bowel syndrome. The response rate was 85% (441/516) for cases and 98% (525/534) for control subjects as of July 1, 2012. The control subjects were frequency matched to the case patients on sex and age (within 10-year categories). As a result, 360 case patients and 400 control subjects were included in the present analysis.

All the study subjects provided written informed consent. Our study was approved by the Ethics Board of Aichi Medical University and by all the participating hospitals.

Data collection

Using a self-administered questionnaire, we collected detailed information on demographic characteristics, medical history, and lifestyle factors. In addition to the questionnaire survey, we obtained a 7-mL venous blood sample from all consenting participants. Genomic DNA was extracted from peripheral lymphocytes and subsequently stored at -30 ℃ until analysis.

Genotyping assays

Genotyping was performed using Fluidigm 192.24 Dynamic Array with BioMark HD Systems and EP1 (Fluidigm Corp., CA). We applied SNPtype assay (Fluidigm Corp., CA) which employs allele-specifically designed fluorescences (FAM or VIC) primers and a common reverse primer. We analyzed the data by the BioMark SNP Genotyping Analysis software to obtain genotype calls. The software defined genotype of each sample based on the relative intensities of fluorescences. The laboratory staff members were blinded to case or control status. Four quality control samples were included in each assay, and the successful genotyping rate was 100%.

Statistical analysis

A χ^2 test was used to test genotype frequencies in control subjects for Hardy-Weinberg equilibrium (HWE) by comparing the observed genotype frequencies with those expected under HWE. The differences in genotype frequencies between cases and controls were also tested using a χ^2 test. Because the biological function of most SNPs has not been clearly defined, a co-dominant genomic model was assumed for SNP effects. We used unconditional logistic regression methods to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between diabetes-associated variants and pancreatic cancer risk. All analyses were adjusted for age (continuous), sex (male or female), BMI (< 20, 20-22.4, 22.5-24.9, or ≥ 25.0), and cigarette smoking (current, former, or never smokers). ORs were also estimated for the risk allele on the basis of a log-additive model.

All *P* values were two-sided, with $P < 0.05$ indicating statistical significance. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for the R program (The R Foundation for Statistical Computing). More precisely, EZR is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

RESULTS

The distribution of genotypes for all SNPs among control subjects did not deviate from HWE. Table 1 shows the selected characteristics of cases and controls. The mean BMI was similar between cases and controls. The number of individuals who had a history of diabetes was 87 (24.1%) in cases and 35 (8.7%) in controls. The OR was 2.95 (95%CI: 1.90-4.57) for those who had a history of diabetes. Individuals who had a BMI of 30 or greater had a 1.21-fold increased risk; however, the association was not statistically significant. The number of ever smokers (including current and former smokers) was 215 (59.7%) in cases and 198 (49.5%) in controls. The SNP profile is summarized in Table 2.

Table 3 shows the associations of pancreatic cancer with individual SNPs in the following genes: *PPARG2*

Table 3 Associations between diabetes-associated single-nucleotide polymorphisms and pancreatic cancer risk

Gene	SNP	Genotype	Case, *n*	Control, *n*	Age- and sex-adjusted OR (95%CI)	[1]Multivariable-adjusted OR (95%CI)
PPARG2	rs1801282	GG + CG	26	27	1.00	1.00
		CC	334	373	0.83 (0.47-1.46)	0.77 (0.43-1.38)
ADIPOQ	rs1501299	AA	19	38	1.00	1.00
		AC	155	167	1.79 (0.98-3.25)	1.71 (0.93-3.15)
		CC	186	195	1.86 (1.03-3.38)	1.85 (1.01-3.39)
CDKAL1	rs2206734	GG	114	138	1.00	1.00
		AG	184	195	1.15 (0.83-1.59)	1.18 (0.85-1.64)
		AA	62	67	1.16 (0.75-1.79)	1.21 (0.78-1.89)
ADRB3	rs4994	TT	228	255	1.00	1.00
		CT	114	131	0.92 (0.67-1.25)	0.88 (0.64-1.21)
		CC	18	14	1.37 (0.66-2.83)	1.36 (0.65-2.87)
KCNQ1	rs2237895	AA	153	156	1.00	1.00
		AC	175	193	0.95 (0.70-1.29)	0.92 (0.67-1.26)
		CC	32	51	0.62 (0.37-1.02)	0.62 (0.37-1.04)
KCNJ11	rs5219	CC	150	159	1.00	1.00
		CT	157	192	0.87(0.64-1.18)	0.90 (0.66-1.24)
		TT	53	49	1.14 (0.72-1.79)	1.19 (0.75-1.90)
TCF7L2	rs7903146	CC	354	394	1.00	1.00
		CT + TT	6	6	1.20 (0.38-3.83)	1.16 (0.36-3.72)

[1]Adjusted for age, sex, body mass index, and cigarette smoking.

(rs1801282), *ADIPOQ* (rs1501299), *ADRB3* (rs4994), *KCNQ1* (rs2237895), *KCNJ11* (rs5219), *TCF7L2* (rs7903146), and *CDKAL1* (rs2206734). With the exception of rs1501299 in the *ADIPOQ* gene ($P = 0.09$), no apparent differences in genotype frequencies were observed between cases and controls. Rs1501299 in the *ADPOQ* gene was positively associated with pancreatic cancer risk; the age- and sex-adjusted OR was 1.79 (95%CI: 0.98-3.25) among those with the AC genotype and 1.86 (95%CI: 1.03-3.38) among those with the CC genotype when compared with individuals with the AA genotype. The ORs remained similar after additional adjustment for cigarette smoking and BMI. Under the log-additive model, each additional copy of risk allele C was associated with a 1.2-fold increased risk of pancreatic cancer (OR = 1.22, 95%CI: 0.96-1.55). In contrast, rs2237895 in the *KCNQ1* gene was inversely related to pancreatic cancer risk, with a multivariable-adjusted OR of 0.62 (0.37-1.04) among individuals with the CC genotype compared with those with the AA genotype. No significant associations were noted for the other 5 SNPs.

DISCUSSION

In this case-control study, we genotyped 7 diabetes-associated genetic polymorphisms, and found that 2 variants in the *ADIPOQ* and *KCNQ1* genes were associated with pancreatic cancer risk in Japanese subjects. The risk variant in the *ADIPOQ* gene had a 1.9-fold increased risk, whereas the risk variant in the *KCNQ1* gene was inversely associated with risk.

Studies examining the association between diabetes-related genetic variants and pancreatic cancer risk were very limited, and the results were inconsistent. In a case-control study examining 15 SNPs in several obesity- and diabetes-related genes, two *FTO* gene variants (rs8050136 and rs9939609) and one *ADIPOQ* gene variant (rs17366743) were positively associated with pancreatic cancer risk; however, these associations were observed only in individuals who were overweight[15].

Of the 37 diabetes risk alleles examined by Pierce *et al*[16], only two SNPs (rs8050136 in *FTO* and rs1387153 in *MTNR1B*) showed significant positive associations with pancreatic cancer risk. However, *ADIPOQ* gene variants were not included in their analyses. We found that rs1501299 in the *ADIPOQ* gene had a positive association with pancreatic cancer risk, with the risk variant CC genotype conferring an approximate 1.9-fold increased risk compared with the AA genotype. The precise mechanism linking this SNP to pancreatic cancer risk is not clear. Adiponectin, which is secreted by adipose tissue, acts as an endogenous insulin-sensitizing hormone[17] and activates intracellular signaling pathways, including AMPK, PPARα, and NF-κB, by binding to two receptors, AdipoR1 and AdipoR2[17]. AdipoR1 has been reported to be upregulated in pancreatic cancer[18]. The adiponectin gene is located on chromosome 3q26, a region associated with susceptibility to the development of type 2 diabetes[19]. Rs1501299 in the *ADIPOQ* gene has been shown to be correlated with adiponectin levels, with the CC genotype exhibiting decreased levels of adiponectin compared with the AA genotype[9,20], Low adiponectin concentrations contribute to insulin resistance, type 2 diabetes, and atherosclerosis[21] as well as obesity-related cancers, including breast and colorectal cancers[22,23]. A prospective study showed that low plasma adiponectin levels are associated with an increased risk of pancreatic cancer, independent of other markers of insulin resistance[24]. Given the essential role of adiponectin in insulin resistance and the strong evidence supporting the positive association of pancreatic cancer with obesity, insulin resistance, and hyperinsulinemia in both

epidemiological and mechanistic studies, it is likely that genetic variations in the adiponectin pathway may affect pancreatic cancer risk through their effects on circulating adiponectin.

KCNQ1 (potassium voltage-gated channel KQT-like subfamily, member 1) encodes the pore-forming subunit of a voltage-gated K+ channel (KvLQT1) and plays a key role in the repolarization of cardiac action potential as a water and salt transporter in epithelial tissues[25]. *KCNQ1* is also expressed in pancreatic islets[26], and a blockade of the channel with the *KCNQ1* inhibitor 293B stimulated insulin secretion[27]. To date, variants in the *KCNQ1* gene exert the greatest effects on the risk of type 2 diabetes in Asians[28]. Of the several SNPs in the *KCNQ1* gene that are associated with increased type 2 diabetes risk in Asians[10,11], we selected rs2237895 because this SNP was reported to be significantly associated with diabetes risk in both GWAS of Japanese people. Furthermore, in a previous study examining the effects of 4 SNPs in the *KCNQ1* gene (rs2237892, rs2283228, rs2237895, and rs2237897) on serum insulin levels following an oral glucose tolerance test in approximately 6000 Scandinavian individuals, only the C risk allele of rs2237895 was associated with reduced insulin release[29]. A 2012 meta-analysis confirmed that the C risk allele of rs2237895 in the *KCNQ1* gene increases the risk of diabetes by 32%[30]. However, we found that the C risk allele of rs2237895 was associated with a decreased risk of pancreatic cancer, which is unexpected and contrary to our hypothesis. This finding may be due to chance, but the mechanisms underlying this inverse association should be explored in further studies.

Diabetes is a complex disease, and susceptibility is determined by both genetic and environmental factors. Additionally, pancreatic cancer develops only in a subset of diabetics. Thus, these factors led us to postulate that certain diabetes-predisposing variants may be associated with a decreased risk of pancreatic cancer. A nested case-control study offered supporting evidence that circulating markers of peripheral insulin resistance, rather than pancreatic β-cell dysfunction, were independently associated with pancreatic cancer risk[31]. This finding, together with our observation of the positive association between rs1501299 in the *ADIPOQ* gene and pancreatic cancer risk, indicates that genetic variations influencing insulin resistance and their impact on circulating biomarkers are closely associated with pancreatic cancer risk.

No significant differences were observed in the genotype distributions between cases and controls in this study, with the exception of rs1501299 in the *ADIPOQ* gene. Other than SNPs in the *ADIPOQ* and *KCNQ1* genes, none of the 5 SNPs we genotyped were associated with pancreatic cancer risk. Among the genes examined in this study, *TCF7L2*, the most significant diabetes-related gene in Western populations, did not show any significant associations in this study. One possible reason for this result is the difference in the minor allele frequency. The very low frequency of *TCF7L2* risk genotypes in this study might make the detection of significant associations difficult. The null association for these SNPs suggests that other causal SNPs in these genes may be involved in pancreatic cancer susceptibility, and further studies are warranted to identify novel risk variants.

Our findings should be interpreted cautiously due to several limitations of this study. First, the results obtained may be due to chance because of the inadequate statistical power or bias inherent in case-control studies. Second, pathology reports were not available for all cases. However, we performed an analysis excluding those cases without pathology reports, and found that the positive association between rs1501299 in the *ADIPOQ* gene and pancreatic cancer remained unchanged. Third, we did not genotype SNPs that have been shown to be related to diabetes-related quantitative traits, including fasting plasma glucose, insulin, and homeostasis model assessment of β-cell function (HOMA-β). These biomarkers have been shown to be associated with pancreatic cancer risk in previous prospective studies[32,33]. Fourth, we did not examine serum levels of adiponectin in this study. Additional studies are necessary to clarify the effects of genetic polymorphisms on serum levels of adiponectin and evaluate their roles in the development of pancreatic cancer. Finally, we cannot exclude the possibility that the observed SNPs are in linkage disequilibrium with causal variants in the same gene or other genes. Further comprehensive analyses of SNPs in the two genes are required to identify the causal variants that confer susceptibility to diabetes or pancreatic cancer.

In summary, the results of our case-control study indicate that rs1501299 in the *ADIPOQ* gene may be associated with pancreatic cancer risk. These findings should be replicated in additional studies.

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COMMENTS

Background

Given the well-recognized, positive association between type 2 diabetes and pancreatic cancer risk in epidemiological studies, it may be interesting to examine whether diabetes-related genetic variants may also be associated with pancreatic cancer risk.

Research frontiers

Although it is likely that a common genetic background predisposes individuals to developing both diabetes and pancreatic cancer, very few molecular epidemiologic studies have addressed this issue.

Innovations and breakthroughs

This case-control study indicates that rs1501299 in the *ADIPOQ* gene may be associated with pancreatic cancer risk in Japanese subjects.

Applications

Genetic variations in the adiponectin pathway may affect pancreatic cancer risk

through their effects on circulating adiponectin. Further comprehensive analyses of SNPs in this gene are required to identify the causal variants that confer susceptibility to diabetes or pancreatic cancer.

Terminology

Single-nucleotide polymorphisms (SNP) are the most common type of genetic variation among individuals. Some SNPs have been linked to increased susceptibility to disease.

Peer review

Very few molecular epidemiologic studies have addressed the issue about the common genetic background which predisposes individuals to developing both diabetes and pancreatic cancer. This a good case-control study, try to examine whether diabetes-related genetic variants are associated with pancreatic cancer risk in Japan. Seven diabetes-related genetic variants were therefore genotyped and it was found that rs1501299 in the *ADIPOQ* gene may be associated with pancreatic cancer risk, although the role of adiponectin variants has not been clarified yet.

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