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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RESEARCH ARTICLE

Lack of Associations between Genetic Polymorphisms in GSTM1, GSTT1 and GSTP1 and Pancreatic Cancer Risk: A Multi-Institutional Case-Control Study in Japan

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Abstract

Background: We aimed to evaluate the role of genetic polymorphisms in tobacco carcinogen-metabolizing genes and their interactions with smoking in a hospital-based case-control study of Japanese subjects. Materials and Methods: We examine the associations of pancreatic cancer risk with genetic polymorphisms in GSTM1, GSTT1 and GSTP1, phase II enzymes that catalyze the conjugation of toxic and carcinogenic electrophilic molecules. The study population consisted of 360 patients and 400 control subjects, who were recruited from several medical facilities in Japan. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between genotypes and pancreatic cancer risk. Results: Among the control subjects, the prevalence of the GSTM1-null genotype and the GSTT1-null genotype was approximately 56% and 48%, respectively. Cases and controls were comparable in terms of GSTM1 and GSTT1 genotype distributions. Neither of the deleted polymorphisms in GSTM1 and GSTT1 was associated with the risk of pancreatic cancer, with an age- and sex-adjusted OR of 0.99 (95%CI: 0.74-1.32) for the GSTM1-null genotype, and 0.98 (95%CI: 0.73-1.31) for the GSTT1-null genotype. The OR was 0.97 (95%CI: 0.64-1.47) for individuals with the GSTM1 and GSTT1-null genotypes compared with those with the GSTM1 and GSTT1-present genotypes. No synergistic effects of smoking or GST genotypes were observed. Conclusions: Our results indicate no overall association between the GSTM1 and GSTT1 deletion polymorphisms and pancreatic cancer risk in the Japanese subjects in our study.

Keywords: GSTM1 - GSTT1 - GSTP1 - pancreatic cancer - risk

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Introduction

The etiology of pancreatic cancer remains largely unknown. Epidemiologic studies have consistently shown positive associations between pancreatic cancer with cigarette smoking and long-standing diabetes (Duell, 2012; Ben et al., 2011). According to a 2008 meta-analysis, current smokers had approximately double the risk of pancreatic cancer relative to nonsmokers (Iodice et al., 2008). Although the exact mechanisms underlying the smoking-pancreatic cancer association remain to be clarified, similar to tobacco-induced cancers, a DNA adduct is thought to play a crucial role in pancreatic carcinogenesis. The accumulation of unrepaired genetic mutations due to tobacco-derived carcinogen-DNA adducts can cause disruption of cell cycle checkpoints

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and chromosomal instability (Hecht, 2008).

The process of carcinogen metabolism involves phase I metabolic activation and phase II detoxification [5], with a variety of enzymes involved in each phase. Cytochrome 450 (CYP1A1) is a phase I enzyme that initiates metabolic activation of carcinogens (Nebert et al., 2006). Glutathione S-transferases (GSTs) are the principal phase II enzymes that catalyze the conjugation of toxic and carcinogenic electrophilic molecules (Hayes et al., 2005). Three common polymorphisms in the GSTM1, GSTT1 and GSTP1 genes have been extensively studied in molecular epidemiologic studies due to their varied effects on enzyme activity (Moyer et al., 2007; Moyer et al., 2008). Variants in the GSTM and GSTT1 genes have attracted the most attention because inherited homozygous deletions of the GSTT1 and GSTM1 genes lead to an absence of enzyme activity, therefore increasing disease susceptibility. For GSTP1, a single nucleotide substitution (A→G) at position 313 of the GSTP1 gene (rs1695) substantially diminishes GSTP1 enzyme activity (Moyer et al., 2006). Loss-of-function (LoF) deletion polymorphisms in the GSTM1 and GSTT1 genes have been linked to an increased risk for many cancers, including head and neck, lung, liver, colon and pancreatic cancers (Geisler et al., 2001; Moore et al., 2005; White et al., 2008; Carlsten et al., 2008; Cote et al., 2009; Jang et al., 2012). Compared with GSTT1 and GSTM1 genetic polymorphisms (Bartsch et al.,1998; Liu et al., 2000; Duell et al., 2002; Jiao et al., 2007; Vrana et al., 2009; Jang et al., 2012), very few studies have studied GSTP1 genetic polymorphisms and their associations with pancreatic cancer risk. Only one previous study reported that GSTP1 polymorphisms were significantly associated with pancreatic cancer survival (Jiao et al., 2007)

Because the frequencies of GST genotypes vary across populations and ethnicities (Di Pietro et al., 2010), large inter-individual differences might exist in the metabolic response to carcinogen exposure. As a result, the risk of pancreatic cancer could be partly determined by these factors. In this study, we examined the associations of pancreatic cancer risk with genetic polymorphisms in GSTM1, GSTT1 and GSTP1 in Japanese subjects, using a hospital-based case-control study.

Materials and Methods

Study subjects

We aimed to clarify the roles of genetic polymorphisms and gene-environment interaction in the development of pancreatic cancer, using data obtained from an ongoing multi-institutional case-control study. The details of our case-control study have been described elsewhere (Lin et al., 2013). Briefly, the eligible cases were patients who were newly diagnosed with pancreatic cancer at five hospitals from April 1, 2010, through May 15, 2012. Imaging modalities and pathologic reports (if available) were used for pancreatic cancer diagnosis. During the same time period, we enrolled control subjects from the following three sources: 1) inpatients and outpatients from the same participating hospitals where the cases were enrolled; 2) relatives of inpatients from the same participating hospitals where the cases were enrolled; and 3) individuals who were undergoing medical checkups at one of the participating hospitals. All of the control subjects who were recruited from among inpatients and outpatients had no prior diagnoses of cancer at the time of enrollment. The diagnoses for control subjects included a variety of diseases, such as anemia, gastric ulcers and irritable bowel syndrome. We achieved a response rate of 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 by sex and age (within 10-year categories). As a result, the data from 360 case patients and 400 control subjects were included in the present analysis.

We obtained written, informed consent from all of the study subjects. The ethical board of Aichi Medical University and all of the participating hospitals approved this study.

Data collection

The study participants completed a self-administered questionnaire covering information on demographic characteristics, medical history and lifestyle factors, such as cigarette smoking, alcohol consumption and dietary intake. Information on cigarette smoking included smoking status (never, former or current smokers), average number of cigarettes smoked per day, age at starting and quitting smoking and duration of smoking. In addition to lifestyle information, a 7-mL venous blood sample was collected from all of the consenting participants.

Genomic DNA was extracted from peripheral lymphocytes in the blood at SRL Hachioji Laboratory and was stored at -30°C until genotyping.

Genotyping assays

All of the genotyping was conducted in the laboratory of Aichi Cancer Center Research Institute in Nagoya, Japan, with the laboratory staff blinded to case or control status. For GSTM1 and GSTT1, the genotyping was performed using the Taqman SNP Genotyping assay. Two quality control samples were included in each assay. The assays were undertaken independently using 30-50 ng of genomic DNA in a 10-μL reaction. The reactions were performed in a 96-well plate format. The GSTM1 and GSTT1 real-time assays were conducted using 4 μL of the 2×Genotyping Master Mix Universal (ABI). The thermocycling conditions were as follows: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles 95°C for 15 seconds and 56°C for 1 minute and 30 seconds. Real-time fluorescence was monitored during PCR amplification, and the results were analyzed using Applied Biosystems 7500 Real-Time PCR systems. The GSTP1 rs1695 polymorphism was genotyped using Fluidigm SNPtype assays.

Statistical analysis

Deviation from Hardy-Weinberg equilibrium (HWE) in the control subjects was evaluated using the chi-squared test. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between GST genotypes and pancreatic cancer risk. All of the analyses were adjusted

for age (continuous) and sex (male or female). The interaction of genotype and smoking with regard to pancreatic cancer risk was assessed using a likelihood ratio test.

The statistical tests were two-sided, and a P-value less than 0.05 was considered statistically significant. All of the statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Table 1 shows the distributions of selected characteristics and risk factors for pancreatic cancer. The cases were more likely to be current smokers and to have a history of diabetes, compared to the controls. The OR was 2.86 (95%CI: 1.79-4.57) for current smokers after adjustment for age, sex, BMI and history of diabetes. Subjects who reported a history of diabetes had a 2.9-fold increased risk of pancreatic cancer (OR=2.94; 95%CI: 1.90-4.57).

As shown in Table 2, the prevalence of the GSTM1-null genotype and GSTT1-null genotype among the control subjects was approximately 56% and 48%, respectively. The cases and controls were comparable

Table 1. Characteristics of Case Patients and Control Subjects

Characteristics	Case patients (N=360)	Control subjects (N=400)	OR (95% CI)
Mean age±SD	67.8±8.8	64.8±9.5	
Male (%)	215 (59.7)	226 (56.5)	
Body mass index (kg/m^2)			
<25	278 (77.2)	312 (78.0)	1.00
25.0-29.9	64 (17.8)	75 (18.7)	0.96 (0.65-1.43)
≥30	16 (4.4)	12 (3.0)	1.21 (0.53-2.77)
Unknown	2 (0.6)	1(0.3)	
Smoking status			
Non-smokers	145 (40.2)	202 (50.5)	1.00
Former smokers	119 (33.1)	140 (35.0)	1.23 (0.82-1.85)
Current Smokers	96 (26.7)	58 (14.5)	2.86 (1.79-4.57)
History of diabetes			
No	269 (74.7)	362 (90.5)	1.00
Yes	87 (24.2)	35 (8.7)	2.94 (1.90-4.57)
Unknown	4 (1.1)	3 (0.8)	

*OR; odds ratio, CI; confidence interval, SD; standard deviation, OR was adjusted for sex, age, body mass index, history of diabetes, and cigarette smoking. The numbers shown in parentheses are percentages

Table 2. Association of Pancreatic Cancer with GSTM1 and GSTT1 Polymorphisms

	Case patients (n=360)	Control subjects (n=400)	OR (95% CI)
GSTM1			
Present	160 (44.4)	175 (43.8)	1.00
Null	200 (55.6)	225 (56.2)	0.99 (0.74-1.32)
GSTT1			
Present	193 (53.6)	209 (52.3)	1.00
Null	167 (46.4)	191 (47.7)	0.98 (0.73-1.31)
*GSTP1 (rs1695)			
AA	266 (73.9)	284 (71.0)	1.00
AG	88 (24.4)	113 (28.3)	0.83 (0.60-1.16)
GG	6 (1.7)	3 (0.7)	2.41 (0.58-9.98)
AG+GG	94 (26.1)	116 (29.0)	0.87 (0.63-1.20)

**OR: odds ratio; CI: confidence interval, OR was adjusted for age and sex

Table 3. Joint Efects of GSTT1, GSTM1 Genotypes on Pancreatic Cancer Risk

GSTT1	GSTM1	Case patients	Control subjects	OR (95%CI)
Present	Present	85	87	1.00
Present	Null	108	122	0.95 (0.64-1.42)
Null	Present	75	88	0.93 (0.60-1.45)
Null	Null	92	103	0.97 (0.64-1.47)
				P for interaction=0.62

*OR: odds ratio; CI: confidence interval, OR was adjusted for age and sex

Table 4. Joint Effects of Smoking and GSTT1, GSTM1 Genotypes on Pancreatic Cancer Risk

GSTT1	Smoking	Case patients	Control subjects	OR (95%CI)
Present	Non-smokers	71	103	1.00
Present	Current smokers	47	28	3.22 (1.72-6.04)
Null	Non-smokers	74	99	1.08 (0.70-1.67)
Null	Current smokers	49	30	3.27 (1.76-6.06)
				P for interaction=0.79
GSTM1				
Present	Non-smokers	67	98	1.00
Presnt	Current smokers	44	23	3.67 (1.93-6.98)
Null	Non-smokers	78	104	1.08 (0.69-1.68)
Null	Current smokers	52	35	2.92 (1.63-5.25)
				P for interaction=0.39

*OR: odds ratio; CI: confidence interval, OR was adjusted for age and sex

in terms of GSTM1 and GSTT1 genotype distributions. Neither of the deleted polymorphisms in GSTM1 and GSTT1 was significantly associated with the risk of pancreatic cancer, with an age- and sex-adjusted OR of almost 1.0. The results remained unchanged after further adjustment for BMI, history of diabetes and cigarette smoking (data not shown). The distribution of GSTP1 rs1695 genotypes among the control subjects deviated from HWE (p=0.02). No significant associations were observed between rs1695 genotypes in GSTP1 and the risk of pancreatic cancer. Compared with individuals with the AA genotype, the age- and sex-adjusted OR was 0.87 (95%CI: 0.63-1.20) among those with the AG and GG genotype.

Table 3 shows the combined effects of GSTM1 and GSTT1 polymorphisms on pancreatic cancer risk. The OR was 0.97 (0.64-1.47) for individuals with the GSTM1 and GSTT1-null genotypes compared with those with the GSTM1 and GSTT1-present genotypes. No statistically significant interactions were noted (P=0.62). No synergistic effects of smoking or GST genotypes were observed (Table 4). The risk estimates were similar for current smokers with the GSTT1 or GSTM1-null genotypes, compared to current smokers with the GSTT1 or GSTM1-present genotypes.

Discussion

We evaluated the associations between genetic polymorphisms in GSTM1 and GSTT1 and pancreatic cancer risk in Japanese subjects. We found that neither the GSTM1-null genotype nor the GSTT1-null genotype was associated with increased pancreatic cancer risk. Furthermore, although smoking was significantly

associated in our study with an increased risk of pancreatic cancer, the results of the gene-environment interactions did not indicate a synergistic effect of smoking and GST-null genotypes on the risk.

The frequencies of GSTM1 and GSTT1-null genotypes vary widely across ethnicities. It has been shown that Asians and Caucasians display higher frequencies of GSTM1-null genotypes than African populations (Di Pietro et al., 2010). The prevalence of the GSTT1-null genotype is low in Caucasians, and it is significantly greater in Asian populations. In our control group, the GSTT1-null genotype represented approximately 48% of the subjects, which was greater than the percentage reported in Caucasians (Di Pietro et al., 2010). The allele frequencies for GSTT1 and GSTM1 in our study were similar to those reported in other Asian populations (Di Pietro et al., 2010).

Previous studies have yielded mixed results regarding the associations between GSTT and GSTM polymorphisms and pancreatic cancer. To date, at least six case-control studies have addressed this association (Bartsch et al., 1998; Liu et al., 2000; Duell et al., 2002; Jiao et al., 2007; Vrana et al., 2009; Jang et al., 2012). All the studies were conducted in Western countries, with the exception of a population-based case-control study in the San Francisco Bay area, in which a small number of Asian participants were included (Duell et al., 2002). No main effects of the GSTT1 and GSTM1-null genotypes on pancreatic cancer risk were noted in any of the studies, with the exception of a population-based case-control study conducted in Canada (Jang et al., 2012).

The lack of association between LoF variants, such as GSTM1 and GSTT1, and pancreatic cancer risk indicates that a common gene-disrupting variant alone might not confer major susceptibility. Two possibilities exist. First, given the high prevalence of null genotypes, such as GSTT1 and GSTM1, it is unlikely that any major effects exist, because natural selection is expected to prevent the most severely deleterious alleles from reaching high population frequencies (MacArthur et al., 2012). Another possibility is that the pancreas is not directly exposed to tobacco-derived carcinogens, suggesting that the effect of carcinogen-metabolizing enzymatic activity might be weaker than in other organs that are directly exposed to tobacco carcinogens, such as the lungs. Even for lung cancer, a meta-analysis of the GSTM1-null genotype showed a weakly positive association, with a summary OR of 1.22 (95%CI: 1.14-1.30) (Carlsten et al., 2008).

On the basis of a multiplicative interaction model, we observed no synergistic effect of smoking and GST-null genotypes on pancreatic cancer risk. Although the notion that smokers with GSTT1 or GSTM1-null genotypes had the highest risk compared with non-smokers with GSTT1 or GSTM1-present genotypes is biologically plausible, the clarification of genotype-environment interactions remains a challenge. This is due to a limited sample size and difficulty of obtaining accurate exposure information. In the population-based case-control study carried out in six San Francisco Bay areas, the OR was 5.0 (95%CI 1.8-14.5) for heavy smokers who had a deletion polymorphism in GSTT1, suggesting that inherited deletion polymorphisms in GSTT1 increase the susceptibility to smoking-related pancreatic cancer (Duell et al., 2002). The results, however, might have been due to chance because they were based on a very limited sample size.

We recognize several limitations of our study. First, as with other case-control studies, selection bias was an inherent limitation and should be addressed when interpreting the study results. Selecting an appropriate control group remains a major challenge, especially in hospital-based case-control studies. Ideally, the cases and controls should come from the same source population. However, the hospital controls did not necessarily represent the same population from which the cases were derived. The frequency of GST genotypes observed among the control subjects in this study was comparable to that obtained from other Asian populations, suggesting that our results regarding GST polymorphisms and pancreatic cancer were robust. Second, we were limited to detecting significant gene-environment interactions in the subgroups. For example, the numbers of cases and controls were small, especially after stratification by smoking status. Third, the genotyping methods, based on PCR techniques, used in our study and in other studies could not distinguish GSTM1 and GSTT1 homozygous wild-type +/+ from heterozygous +/- individuals. Only one previous study found phenotypic differences between these two groups based on a newly developed assay (Moore et al., 2005). Fourth, although we showed that a combination of GSTM1 and GSTT1-null genotypes was not associated with the risk of pancreatic cancer, the pathways of carcinogen metabolism are complex and are mediated by a variety of factors. These factors include the balance between metabolic activation and the detoxification of tobacco carcinogen compounds, as well as the efficiency of DNA repair. For example, it is likely that a deficiency in one class of GST enzymes due to a genetic polymorphism can be compensated for by the presence of other classes of GST enzymes. We genotyped rs1695 in GSTP1 and found no significant association between rs1695 polymorphisms and the risk of pancreatic cancer. It should be noted that the distribution of genotypes among control subjects was not in HWE. The reason for this fact is unclear, but genotyping error and population stratification are possible explanations (Pompanon et al., 2005). Further studies are needed to integrate genetic variations into different pathways, to define the risk of pancreatic cancer better.

In conclusion, our case-control study indicated no overall association between the GSTM1 and GSTT1 variants and pancreatic cancer risk in Japanese subjects. As common low-risk variants in different genes might act collectively to confer susceptibility to pancreatic cancer, further studies will be required to uncover the full spectrum of these variants and their effects on pancreatic cancer.

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Original Article

Gastroduodenal stenting with Niti-S stent: Long-term benefits and additional stent intervention

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Background and Aim: Self-expandable metallic stents have mainly been used for the palliation of malignant gastric outlet obstruction (GOO). However, their use in long-term survivors and the feasibility, safety and benefit of additional intervention for stent dysfunction remain controversial. The present study examined the long-term benefits of endoscopic gastroduodenal stenting.

Methods: We reviewed 61 patients treated with Niti-S stents at several hospitals and estimated the efficacy of stent intervention, stent patency, eating period and factors related to poor effectiveness.

Results: All 61 first stent interventions and 14 additional stent interventions (11 second interventions and 3 third interventions) were successfully carried out. Clinical success rates were 83.6% and 85.7%, and median stent patency was 214 days and 146 days ($P = 0.47$), respectively. Fifty patients could be treated with a first stent only, and 11 patients received additional stents. At the time of study termination or death, 70.0% of the former group and 63.6% of the latter group maintained oral intake ($P = 0.71$), and each 86% and 100% among the group could maintain oral intake for a period exceeding half of their remaining lives after first stent intervention. Karnofsky performance status ≤50 ($P = 0.03$), ascites ($P = 0.009$), and peritoneal dissemination ($P = 0.001$) appeared to be factors related to poor effectiveness.

Conclusions: Despite the presence of factors related to poor effectiveness, endoscopic gastroduodenal stenting would be the first treatment of choice for GOO and provide long-term benefits. If stent dysfunction occurs, additional stent intervention enables continued oral intake safely.

Key words: additional stent intervention, factors related to poor effectiveness, gastric outlet obstruction (GOO), long-term benefit, Niti-S gastroduodenal stenting

INTRODUCTION

FOR PALLIATION OF symptomatic malignant gastric outlet obstruction (GOO), endoscopic gastroduodenal stent intervention is an effective and minimally invasive procedure, and has become an alternative to surgical gastroenterostomy.[1–3] With the availability of through-the-scope (TTS)-type stents, stent placement has become technically easier and more widespread.[4,5]

The Niti-S Pyloric Duodenal D-type stent (Niti-S stent; Taewoong Medical, Seoul, Korea) is an uncovered nitinol stent, and has unique features of low axial force, little foreshortening, and high expansible force. We use the Niti-S stent to palliate most of our patients with malignant GOO. However, only a few published reports on Niti-S gastroduodenal stenting exist.[6,7] Some studies have reported that the long-term outcome of endoscopic stent intervention is less favorable compared with surgical gastroenterostomy.[8,9] In our experience, many patients experience long-term survival after endoscopic gastroduodenal stenting, with continued palliation with additional stent interventions among those with stent dysfunction. However, the use of this approach in patients with long expected survival remains controversial.[10]

Estimating first and additional gastroduodenal stent interventions, the present study aimed to determine the feasibility, efficacy and benefit of long-term palliation of malignant GOO using Niti-S stents.

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METHODS

Patients

WE RETROSPECTIVELY STUDIED a total of 61 patients with symptomatic malignant GOO who received initial treatment with a Niti-S Stent from March 2011 to April 2013. Patients were enrolled at two tertiary medical centers in Japan: Aichi Cancer Center Hospital and Yokohama City University Hospital. All patients were assessed clinically, and all malignancies were staged with standard cross-sectional imaging, including computed tomography (CT) before stenting. We excluded patients who had been treated with stents other than Niti-S, those with prior surgical resection of the stomach or duodenum, and patients with multiple levels of obstruction in the intestinal tract.

Equipment and procedure

We used uncovered Niti-S stents of 6 cm, 8 cm, 10 cm, and 12 cm lengths, with fully expanded diameters of 20 mm or 22 mm. The 10-Fr delivery system enabled through-the-scope stent placement using the TJF-260V, JF-260V, CF-H260AI, and CF-Q240 (Olympus, Tokyo, Japan) endoscopes with a working channel diameter of ≥3.7 mm.

Patients were sedated with i.v. pethidine hydrochloride and midazolam. After identifying the site and length of obstruction with a water-soluble radiographic contrast medium using endoscopic retrograde cholangiography (ERCP) catheter (Tandem XL; Boston Scientific Japan, Tokyo, Japan), the stenosis was negotiated with a biliary guidewire (Jagwire; Boston Scientific Japan or RevoWave-J; Piolax Medical Devices, Inc., Kanagawa, Japan). Without pre-dilation, the stent delivery system was inserted over the guidewire through the working channel and deployed across the stricture with fluoroscopic guidance.

After stent intervention and follow up

One day after stent intervention, stent position and expansion were assessed with abdominal X-rays in all patients. If there was no abdominal pain, oral intake was allowed starting with liquids, followed by semisolids and then solids. If the patients' nutritional status improved sufficiently, they were considered for chemotherapy and/or radiotherapy, as appropriate. The primary physician followed up patients for symptom resolution until study termination (July 2013), or death.

The Gastric Outlet Obstruction Scoring System (GOOSS) was used before and after stent intervention for assessing symptomatic status of patients.[11] This score divides oral intake ability into the following categories: 0, no oral intake; 1, liquids only; 2, soft solids; 3, low-residue diet; and 4, normal diet. In the present paper, score 4 is our original score.

Outcomes

Primary outcome was long-term improvement in oral intake as estimated by eating period. Secondary outcomes were feasibility and safety of additional stent interventions, and factors related to poor effectiveness.

We compared the following factors between patients who were palliated with a single stent, and those who required additional stenting: technical success, procedure time, time to oral fluids and solids, length of hospitalization, oral intake ability, clinical effect, stent patency, stent dysfunction, and complications. The clinical effect was assessed 1 week after any interventions and classified into three levels: good, improvement of both oral intake and symptoms; moderate, improvement of either oral intake or symptoms; poor, improvement of neither oral intake nor symptoms. Early and late complications were defined as those occurring within 1 week, and later than 1 week after the procedure, respectively. The following factors were studied for prediction of poor stent efficacy: age, sex, site of obstruction, tumor stage, pre-intervention GOOSS, Karnofsky performance status (KPS), previous gastroduodenal or biliary stenting, ascites and peritoneal dissemination. We differentiated ascites from peritoneal dissemination by the presence of peritoneal nodules in the latter condition, with or without ascites, as detected by imaging modalities.

To estimate long-term outcome, patients with first-stent intervention only were compared to patients with additional stent intervention according to the following: final oral intake ability, eating period, survival time after first stent intervention, and percent eating period. Eating period and survival time were defined as the time from the date of the first stent intervention to the final follow up or death, respectively. We also reviewed post-procedure therapy and biliary drainage.

The present study was approved by the ethical committees of Aichi Cancer Center Hospital (3–145) and Yokohama City University Hospital (B140109014) and registered as a clinical trial (UMIN000012784).

Statistical analysis

Summary statistics are presented as means ± standard deviation for parametric data and as medians and interquartile range for non-parametric data. Standard statistical comparisons were made as appropriate. Multivariate analysis of factors related to poor effectiveness was done with the logistic regression method. Patency of duodenal stents, cumulative eating period, and cumulative survival of the two groups

were estimated with the Kaplan–Meier method, and the groups were compared using the log–rank test. All results were considered significant at $P < 0.05$. StatMate IV software (ATMS, Tokyo, Japan) was used for all statistical analyses.

RESULTS

Patient characteristics and interventions

MEAN AGE OF the 61 included patients (35 males) was 64.0 ± 10.3 years. Baseline demographics and clinical characteristics are shown in Table 1. Pancreatic cancer was the most common etiology (60.7%), and the site of obstruction was at the duodenum in 93.4% and at the pylorus in 6.6% patients. Twenty-six (42.6%) patients had no oral intake (GOOSS ≤ 0), and 16 patients (26.3%) had a KPS ≤ 50 before stenting.

Table 1 Patient demographics and clinical characteristics ($N = 61$)

Mean age ± SD (years)	64.0 ± 10.3
Sex, *n* (%)	
Male	35 (57.4)
Female	26 (42.6)
Tumor diagnosis, *n* (%)	
Pancreatic cancer	37 (60.7)
Gastric cancer	6 (9.8)
Duodenal cancer	3 (4.9)
Cancer of duodenal papilla	1 (1.6)
Bile duct cancer	9 (14.8)
Gallbladder cancer	2 (3.3)
Metastasis	3 (4.9)
Renal cancer	1 (1.6)
Colon cancer	1 (1.6)
Breast cancer	1 (1.6)
Site of obstruction, *n* (%)	
Pylorus	4 (6.6)
Duodenum, pars I	21 (34.4)
Duodenum, pars II	17 (27.9)
Duodenum, pars III	19 (31.1)
Ability of oral intake (GOOSS)	
Median score, *n* (%) [quartile]	1 [0–1]
0	26 (42.6)
1	25 (41.0)
2	10 (16.4)
Karnofsky performance score	
Median score, *n* (%) [quartile]	60 [60–70]
20–30	2 (3.3)
40–50	14 (23.0)
60–70	39 (63.9)
80	6 (9.8)

GOOSS, gastric outlet obstruction scoring system.
0, no oral intake; 1, liquids only; 2, soft solids; 3, low-residue diet; 4, normal diet.

Clinical outcomes related to stent intervention

Clinical results

Additional stent interventions were required in 14 of 61 cases (11 second stents and 3 third stents). There was no difference in median procedure time between the first stent interventions (First-stent group) and the additional stent interventions (Add-stent group) (21 *vs* 22 min, $P = 0.72$) (Table 2), with 100% technical success rates in both. Median post-procedure length of hospitalization was also similar: 11 days for the First-stent group, and 10 days for the Add-stent group ($P = 0.43$).

Median time to oral fluid intake and oral solids intake was 1 day and 2 days, respectively, in both groups ($P = 0.27$, and 0.09, respectively). Median maximum GOOSS after intervention was 3 ($P = 0.87$), and median recovered GOOSS was 2 in both groups ($P = 0.12$). Clinical effect at 1 week was good, moderate, and poor in 83.6%, 6.6%, and 9.8% in the First-stent group, and 85.7%, 0%, and 14.3% in the Add-stent group, respectively. Median stent patency was 230 days in the First-stent group and 172 days in the Add-stent group ($P = 0.47$) (Fig. 1).

Stent dysfunction and complications

Causes of 15 episodes of initial stent dysfunction were tumor ingrowth in seven, tumor overgrowth in six, and stent migration in two patients. Causes of five repeat stent dysfunctions were tumor ingrowth and stent breakage in one patient each, and tumor overgrowth in three patients (Table 3). Early complications, occurring within 1 week of the procedure, and late complications, occurring 1 week after the procedure, are shown in Table 3. In one case, we detected that the stent was spontaneously disrupted by a CT scan 205 days after the previous intervention. In four cases, bilirubin levels increased 1 day after the procedure, suggesting the possibility that the duodenal stent had accelerated the jaundice.

Factors related to poor effectiveness

Gastroduodenal stent intervention was ineffective for resumption of oral intake and/or symptom relief in 12 cases (Table 4). We examined the following factors for any relationship with poor effectiveness: sex, tumor type, site of obstruction, tumor stage, baseline-GOOSS, KPS ≤ 50, additional stenting, previous biliary stenting, ascites, and peritoneal dissemination. Univariate analysis identified KPS ≤ 50 ($P = 0.03$), ascites ($P = 0.009$), and peritoneal dissemination ($P = 0.001$) as significant factors (Table 4). On multivariate logistic regression analysis, peritoneal dissemination was a

Table 2 Clinical feasibility and efficacy of stent interventions

	First-stent group $N = 61$	Add-stent group $N = 14$	*P*-value
Technical success, *n* (%)	61 (100)	14 (100)	NA
Median procedure time, min [quartile]	21 [18–30]	22 [18–33]	0.72
Median time to oral fluids, days [quartile]	1 [1–1]	1 [1–1]	0.27
Median time to oral solids, days [quartile]	2 [1–3]	2 [1–3]	0.09
Median post-procedure length of hospitalization, days [quartile]	11 [7–22]	10 [7–22]	0.43
Post-procedure oral intake ability			
Median maximum GOOSS [quartile]	3 [3–4]	3 [3–4]	0.87
Median recovered GOOSS [quartile]	2 [1–3]	2 [1–3]	0.12
Clinical effect†, *n* (%)			0.7
Good	51 (83.6)	12 (85.7)	
Moderate	4 (6.6)	0 (0)	
Poor	6 (9.8)	2 (14.3)	

†Good: improvement of both oral intake and symptoms 1 week after intervention.
Moderate: improvement of either oral intake or symptoms 1 week after intervention. Poor: improvement of neither oral intake nor symptoms 1 week after intervention.
GOOSS, gastric outlet obstruction scoring system; NA, not applicable.

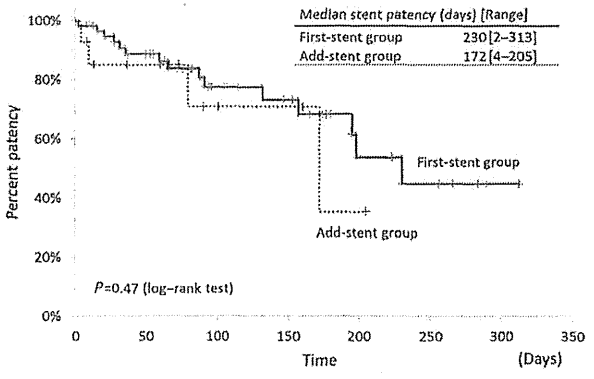

Figure 1 Stent patency is shown with the Kaplan–Meier method. There is no significant difference in median stent patency.

significant independent factor related to poor effectiveness, with an odds ratio of 9.94 (95% confidence interval: 1.82–53.2, $P = 0.01$) (Table 4).

Long-term outcomes of all patients

Long-term outcome was studied in 50 patients with single-stent intervention (First-stent-intervention-only group) and in 11 patients with additional stent intervention (Additional-stent-intervention group). Details of oral intake ability of the patients in the two groups are given in Table 5. Median duration of oral intake, the eating period, was 81 days in the First-stent-intervention-only group and 187 days in the Additional-stent-intervention group, but there was no significant difference ($P = 0.14$). Median survival time after first stent intervention in the Additional-stent-intervention group also tended to be longer than in the First-stent-intervention-only group, but there was no significant difference (First-stent-intervention-only group 94 days *vs* Additional-stent-intervention group 233 days, $P = 0.17$). Most patients could maintain oral intake for a period exceeding half of their remaining lives after first stent intervention (86.0% in the First-stent-intervention-only group and 100% in the Additional-stent-intervention group) (Fig. 2).

Table 3 Stent dysfunction and complications of each stent intervention

	First-stent group $N = 61$	Add-stent group $N = 14$	*P*-value
Stent dysfunction, *n* (%)	15 (24.5)	5 (35.7)	NA
Tumor ingrowth	7	1	
Tumor overgrowth	6	3	
Migration	2	0	
Breakage	0	1	
Early complications, *n* (%)	7 (11.5)	0 (0)	0.18
Stent migration	1	0	
Tumor bleeding	1	0	
Pancreatitis	1	0	
Jaundice	4	0	
Late complications, *n* (%)	7 (11.5)	3 (21.4)	0.32
Stent migration	1	0	
Tumor bleeding	4	0	
Recurrent cholangitis	2	3	

NA, not applicable.

Table 4 Univariate and multivariate analysis of factors related to poor effectiveness in 75 interventions

	Univariate analysis			Multivariate analysis	
	Good $N = 63$	Moderate/poor $N = 12$	*P*-value	Odds ratio	*P*-value
Mean age ± SD, years	63.5 ± 11.0	64.2 ± 10.4	0.53		
Sex					
Male	40	6	0.52		
Female	23	6			
Diagnosis					
Pancreatic cancer	40	4	0.06		
Gastric cancer	6	2			
Duodenal cancer	3	2			
Bile duct cancer	6	4			
Metastasis	3	0			
Other	5	0			
Site of obstruction					
Pylorus	5	2	0.20		
Duodenum, pars I	21	4			
Duodenum, pars II	16	5			
Duodenum, pars III	21	1			
Tumor stage					
Metastatic	41	10	0.32		
Locally advanced	22	2			
Pre-GOOSS ≤0	20	7	0.10		
Karnofsky performance score ≤50	13	6	0.03	1.95	0.39
Additional stent	12	1	0.68		
Biliary drainage before intervention	30	6	1.00		
Ascites	14	7	0.009	2.88	0.18
Peritoneal dissemination	16	9	0.001	9.94	0.01

GOOSS, gastric outlet obstruction scoring system.

Thirty patients in the First-stent-intervention-only group were given chemotherapy and three patients (two with pancreatic cancer and one with duodenal cancer) could be resected surgically together with gastroduodenal stents after chemotherapy. In the Additional-stent-intervention group, seven patients were given chemotherapy after the first stenting (Table 5). During their clinical course, 35 patients in the First-stent-intervention-only group (70.0%) and seven patients in the Additional-stent-intervention group (63.6%) required biliary drainage. Methods of biliary drainage are shown in Table 5.

DISCUSSION

FEATURES OF THE Niti-S stent, such as malleable flexibility with a low axial force and a low rate of foreshortening compared with braided-type stents, means the stent can be placed comparatively easily and safely even across angulated strictures, and is also suitable for additional stenting. Clinical data of the Niti-S stent[4,6,7] and other enteral stents are summarized in Table 6. The Wallstent or WallFlex enteral stents (Boston Scientific Japan) were used early and widely in clinical practice before the Niti-S stent became available. Because of the strong axial force of the Wallstent, the risk of kinking and perforation may be increased when used in tortuous and curved anatomy with reported perforation rates of 0–5%. However, these stents have flared ends to reduce the risk of migration which was reported to occur in 0–2% of patients.[12–20]

Dysfunction of uncovered stents would be caused mainly by tumor ingrowth and tumor overgrowth. A recent study reported that a covered metallic stent was associated with less frequent stent dysfunction >4 weeks after stenting, but there was no significant difference in median stent patency.[21] If stent dysfunction occurred, we considered additional stent intervention as a first rescue method based on each patient's condition and prognosis. However, to date, there have been few studies verifying the utility of additional interventions

Table 5 Final ability of oral intake and follow up of all patients after procedure

	Patients with first-stent intervention only $N = 50$	Patients with additional stent intervention $N = 11$	*P*-value
Final ability of oral intake, *n* (%)			0.71
Solids	35 (70.0)	7 (63.6)	
Liquids only	12 (24.0)	4 (36.4)	
NPO	3 (6.0)	0 (0)	
Median eating period (days) [range]	81 [0–313]	187 [31–324]	0.16
Median post-procedure† survival time (days) [range]	94 [8–419]	233 [39–325]	0.17
Percent eating period ≥50%, *n* (%)	43 (86.0)	11 (100)	0.33
Post-procedure† therapy, *n* (%)			1.00
Chemotherapy	31 (62.0)	7 (63.6)	
Surgical resection after chemotherapy	3	0	
Best supportive care	19 (38.0)	4 (36.4)	
Biliary drainage treatments, *n* (%)	35 (70.0)	7 (63.6)	0.73
EBD	24	2	
EUS-BD	5	3	
PTBD	6	2	

†After first intervention.
EBD, endoscopic biliary drainage; EUS-BD, endoscopic ultrasound-guided biliary drainage; GOOSS, gastric outlet obstruction scoring system; NPO, nothing per oral; PTBD, percutaneous transhepatic biliary drainage.

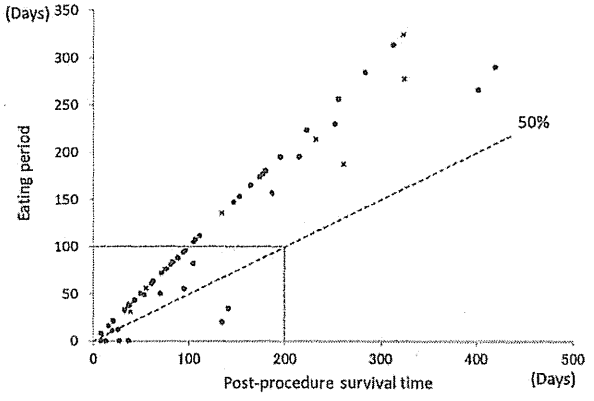

Figure 2 Scatter plot showing eating periods of all patients. Most patients could maintain oral intake for a period exceeding half of their remaining lives after first stent intervention (86.0% in the First-stent-intervention-only group (•) and 100% in the Additional-stent-intervention group (×).

for stent dysfunction.[10] Comparing first stent intervention and additional stent intervention in the present study, clinical feasibility and efficacy were similar with respect to technical success, procedure time, time to oral intake, post-procedure length of hospitalization, improvement of GOOSS, and clinical effect. Technical success and clinical success rates of additional stent intervention were 100% and 85.7%, respectively, which compare favorably with previous reported rates of technical success ranging from 92.9% to 100%, and clinical success ranging from 76.9% to 94.4% (Table 6).[4,6,7,12–20] Overall complication rates are also similar to those from previous studies (from 4% to 36.6%). In the current study, there is no unique complication related to additional stent intervention, which demonstrates the feasibility and safety of additional stent interventions.

We identified the following three features predictive of a suboptimal response to gastroduodenal stenting: KPS ≤ 50, ascites, and peritoneal dissemination. Despite excluding patients with evident distal obstruction of the intestinal tract, presence of peritoneal dissemination was associated with poor clinical effectiveness of gastroduodenal stents. This result was a little different from a previous study which did not find peritoneal dissemination as a negative predictive factor for solid food intake.[22] Although these factors could predict poor effectiveness, we experienced clinical success in more than half of cases even when these factors were present. Hence, in clinical practice, the mere presence of these factors should not be excluded from palliative stenting.[17]

Data from previous studies suggest that bypass surgery should be considered for patients in whom long-term survival >2 months is expected.[8,9] However, recent data also show positive outcomes after stent placement for patients with long survival (Table 6). Hence, the relative indications

Table 6 Comparison of published gastroduodenal stent studies

Study First author	Year	Stent type	No. patients	Technical success *n* (%)	Clinical success *n* (%)	Median eating period (days) [range]	Median survival (days) [range]	Overall complications *n* (%)
Telford *et al.*[12]	2004	Wallstent	176	173 (98.3)	133/159[¶] [83.6]	146[††] [65–202][†]	97 [62–116][†]	14 (8.0)
Graber *et al.*[13]	2007	Wallstent	51	50 (98.0)	43 (84.3)	NR	71.5 [9–515]	17 (33.3)
Maetani *et al.*[6]	2007	Niti-S	37	36 (97.3)	34 (91.9)	NR	118 (NR)	6 (16.2)
van Hooft *et al.*[14]	2009	WallFlex	51	50 (98.0)	43 (84.3)	307[††] [135–470][‡]	62 [35–156][‡]	7 (13.7)
Piesman *et al.*[15]	2009	WallFlex	43	41 (95.3)	NR (75)[‡‡]	NR	49 (NR)	15 (34.9)
Shaw *et al.*[16]	2010	WallFlex	70	65 (92.9)	62 (88.6)	NR	54 [3–570]	6 (8.6)
Maetani *et al.*[4]	2010	Niti-S	53	52 (98.1)	50 (94.3)	NR	88 (NR)	13 (24.5)
Mendelsohn *et al.*[17]	2011	WallFlex or Wallstent	201	192 (95.5)	158 (82.3)	NR	160[§§] [10–1312]	NR (4)
Lee *et al.*[18]	2011	Wallstent	57	57 (100)	52 (91.2)	NR	95 [3–1026]	NR[¶¶]
van Hooft *et al.*[7]	2011	Niti-S	52	50 (96.2)	40 (76.9)	43 [190][§]	82 [31–135][‡]	12 (23.1)
Costamagna *et al.*[19]	2012	WallFlex	206	202 (98.1)	NR (91)	NR	94 [79–112][†]	41 (19.9)
Cha *et al.*[20]	2013	Niti-S, UltraFlex or WallFlex	85	82 (96.5)	68 (80.0)	76.1[a] [18–293]	77.3[a] (NR)	NR
Sasaki *et al.*[5]	2013	WallFlex	42	42 (100)	35 (83.3)	90 [33–129][†]	99 [54–180][†]	11 (26.2)
Present study	2014	Niti-S (first stenting)	61	61 (100)	51 (83.6)	81[b] [0–313]	94[b] [8–419]	14 (23.0)
		Niti-S (additional stenting)	14	14 (100)	12 (85.7)	187[c] [31–324]	233[c] [39–325]	3 (21.4)

[†][95% confidence interval].
[‡][quartile].
[§][maximum no. days].
[¶]Seventeen patients were excluded from the analysis after stent insertion.
[††]Data were calculated from only the patient population who could attain clinical success.
[‡‡]Percentage of patients who attained a GOOSS increase ≥1 seven days after procedure.
[§§]Data were calculated from patients with carcinomatosis.
[¶¶]Publication does not state whether the events occurred with duodenal or colonic stents.
[a]Data are mean no. days.
[b]Data are from patients in the First-stent-intervention-only group.
[c]Data are from patients in the Additional-stent-intervention group.
GOOSS, gastric outlet obstruction scoring system; NR, not reported.
Niti-S stent, Taewoong Medical, Seoul, Korea; UltraFlex stent, Boston Scientific Japan, Tokyo, Japan; WallFlex stent, Boston Scientific Japan; Wallstent, Boston Scientific Japan.

of stenting and surgical bypass need to be redefined. We had patients who achieved long-term survival after first stent intervention and who continued therapy for their malignancy with additional stent interventions. Our data show the median eating period after first stenting was 81 days in the patients with a first stent only, and 187 days in the patients with additional stenting; the median overall survival time in the present patient population was 94 days and 233 days, respectively. The present results of first-stent patents were similar to those from recent studies, whereas additional-stent patients showed relatively longer outcomes than recent studies (Table 6). The good outcomes achieved with additional stenting to maintain patency of the gastric outlet confirm the utility of gastroduodenal stenting among

patients with predicted long survival. As a result, most patients could be given chemotherapy soon after the first procedure because of its lesser invasiveness and safety, which is a substantial advantage over bypass surgery. Moreover, some patients with a good response to chemotherapy underwent surgical resection of their original tumor, which indicates the utility of gastroduodenal stenting for patients with GOO who will undergo neoadjuvant chemotherapy as a 'bridge to surgery'.

Forty-two patients required biliary drainage, of which 34 patients were successfully treated with endoscopic techniques, including endoscopic ultrasound-guided biliary drainage (EUS-BD). There have been only a few studies to determine the feasibility of combined endoscopic biliary and gastroduodenal stenting,[23,24] and it remains controversial which biliary drainage method should be selected.

There are some unique points in our study. Although this study enrolled only patients without prior surgical resection of the stomach or duodenum who were treated with the Niti-S stent only, more than 60 patients and 70 interventions could be evaluated. Previous studies included patients who had obstruction of surgical anastomoses and efferent loops, and had been treated with various types of stent.[4,6,7,12–20] Moreover, we estimated the feasibility and efficacy of additional stent interventions by the comparison between first and additional stent interventions. Therefore, the present study is the first to have accurately evaluated the efficacy of the Niti-S stent for gastroduodenal outlet patency, additional interventions, and factors related to poor effectiveness. All stent interventions were carried out at each center under the supervision of experienced endoscopists; therefore, the data may not be generalized to other patient populations. However, the technical success rate was 100%.

In conclusion, despite the presence of factors related to poor effectiveness, endoscopic gastroduodenal stenting would be the first treatment of choice for GOO without multiple levels of obstruction as it provides long-term benefits independently of patient prognosis. If stent dysfunction occurs, additional stent intervention would enable safe continued oral intake, and more patients could obtain long-term benefits.

CONFLICT OF INTERESTS

AUTHORS DECLARE NO conflict of interests for this article.

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Editorial

Is endoscopic ultrasonography-guided biliary drainage really that wonderful?

RECENTLY, INTERVENTIONAL ENDOSCOPIC ultrasonography (EUS) has become remarkably popular, especially for EUS-guided biliary drainage (EUS-BD). Although several authors have reported the usefulness and safety of EUS-BD, to date, relatively few prospective studies and large-scale case reports have been conducted. Almost all papers have reported the efficacy, safety and high success rate of EUS-BD. EUS-BD enables easy access of the biliary tract, even in postoperative patients with altered anatomy or digestive tract obstruction. Whereas many authors refer to EUS-BD as a single entity, EUS-BD includes several procedures, such as EUS-guided choledochoduodenostomy (EUS-CDS), hepaticogastrostomy (EUS-HGS), EUS-rendezvous and EUS-antegrade drainage. Hence, these procedures need to be distinguished from each other to accurately evaluate them. In particular, EUS-CDS is quite different from EUS-HGS. Most papers about EUS-BD are reported from high-volume centers and by skillful endoscopists. Yet, is EUS-BD really that easy? Park *et al.* reported that the success rates of EUS-HGS and EUS-CDS were 100% (31/31) and 92% (24/26), respectively.[1] In two prospective studies, we reported a success rate of EUS-CDS of 94% (34/36).[2,3] According to these studies, EUS-BD seems to be an easy technique with a high success rate. However, contrary to these results, a Spanish national survey reported that EUS-BD, especially EUS-HGS and EUS-rendezvous, does not have such a high success rate and is sometimes technically difficult in comparison with EUS-CDS.[4] They reported success rates with EUS-HGS, EUS-CDS and EUS-rendezvous of 64.7%, 86.3% and 68.3%, respectively. However, most institutions in the Spanish survey were not such high-volume centers, with an average of <20 procedures in total. Their results seem to provide more realistic clinical data from general hospitals.

The technical difficulties with EUS-HGS include puncture of the small branch bile duct (B3) and guidewire maneuvering to the upstream part of the main bile duct. Conversely, if the bigger main bile duct is to be punctured, although maneuvering the guidewire to the hepatic hilum is easy, there is a high risk of blockage of the bile stream after insertion of the covered metal stent. When we puncture a dilated bile duct in the left hepatic lobe for EUS-HGS, we can easily puncture B2 compared with B3. However, usually B2 is punctured through the esophagus, with the consequently higher risk of mediastinitis. Hence, we should avoid puncturing B2 and instead opt for puncture of B3 via the stomach. The technical difficulties of EUS-CDS are related to dilatation of the puncture route. An electric dilator can easily resolve this problem. If the puncture site is near the hepatic hilum, we should avoid blocking the hepatic duct with a covered metal stent and instead use a plastic stent. With the rendezvous technique, guidewire maneuvering is sometimes difficult, especially while attempting to manipulate a stricture. Moreover, EUS-rendezvous is not easy to carry out and has a low success rate, particularly the transhepatic route.

The high risk of complications associated with EUS-BD is its biggest problem. Most papers have reported early complication rates of 10–30%, although late complications are rare. The rendezvous technique is not an exception. While it is commonly believed that the EUS-rendezvous technique has a low complication rate, this is, in fact, not true.[4] EUS-HGS is the most challenging of the EUS-BD procedures with a high complication rate, with one case fatality as a result of stent migration into the abdominal cavity already being reported.[5] This indicates that even if stent placement is successfully accomplished, the possibility of stent migration immediately or a few days later still remains. As there is some distance between the stomach and the liver, and these two organs are not adjacent to each other, stent migration occurs easily. Furthermore, although severe complications, such as internal stent migration, have not been published as a result of publication bias, in fact, according to medical meetings and our experience of stent migration cases, some severe complications resulting from EUS-HGS have, indeed, occurred. Bile peritonitis is also a common complication of all EUS-BD procedures. Bile peritonitis is usually, however, not severe and can be treated conservatively. Additionally, its occurrence can be minimized by using a metal stent, as in EUS-CDS.[2]

What about the clinical course following EUS-BD? We previously reported prolonged bile duct patency and a good out come with EUS-CDS.[2,3] If there is progression of duodenal obstruction, double stenting (EUS-CDS plus duodenal stent) can be done, which also has a high functional success rate. If anything, the clinical course of EUS-CDS is probably better than that of transpapillary drainage. However, the clinical course of EUS-HGS is not always good. This is