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Diagnostic yield of the plasma free amino acid index for pancreatic cancer in patients with diabetes mellitus

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ABSTRACT

Objectives: A multivariate index calculated using plasma free amino acids (PFAA index) was reported as a diagnostic biomarker for pancreatic cancer (PaC). Although diabetes mellitus (DM) is expected to be an early diagnostic indicator of PaC, identifying the high-risk individuals among patients with DM is warranted. We evaluated the diagnostic yield of the PFAA index for PaC in patients with DM.
Methods: We compared the diagnostic yield of the PFAA index between individuals with and those without DM. Cases and controls were recruited prospectively, and controls were matched to cases at a 1:1 ratio for age, sex, and DM status.
Results: A total of 180 case–control pairs were included in the analysis. The prevalence of DM was 53.3%. The sensitivity of the PFAA index was 66.7% in cases with DM and 56.0% in those without DM ($P = 0.14$), and the specificity was 92.7% in controls with DM and 94.0% in those without DM ($P = 0.95$).
Conclusions: This matched case-control study revealed a comparable diagnostic yield of the PFAA index for PaC in individuals with and those without DM. The PFAA index can be used as a biomarker for further diagnostic imaging in selected patients with DM.

Introduction

Pancreatic cancer (PaC) has a dismal prognosis, with a 5-year survival rate of <8% [1]. We previously reported that patients diagnosed with PaC at the asymptomatic stage have a better prognosis than those diagnosed at the symptomatic stage [2]. A screening program for PaC is warranted in asymptomatic individuals to improve the prognosis of PaC.

A number of diagnostic biomarkers have been investigated as screening tools for PaC [3–6]. Plasma free amino acid (PFAA) profiles in patients with PaC are characterized by elevated levels of serine (Ser) and isoleucine (Ile) and a decreased level of histidine (His). By comparing the PFAA profiles between patients with PaC and healthy controls, a multivariate index consisting of the concentrations of six PFAAs (Ser, Ile, His, asparagine [Asn], alanine [Ala], and tryptophan [Trp]; PFAA index) was developed using logistic regression analysis as a biomarker for diagnosing PaC [7].

The prevalence of PaC is relatively low, so screening of a mass population is impractical in terms of cost effectiveness. Diabetes mellitus (DM) is a well-known risk factor for PaC [8,9]; thus, applying the PFAA index to patients with DM is expected to be useful for PaC screening. However, the concentrations of PFAAs are reportedly altered in patients with DM [10,11]. Therefore, in this study, we investigated the diagnostic yield of the PFAA index for PaC in patients with DM.

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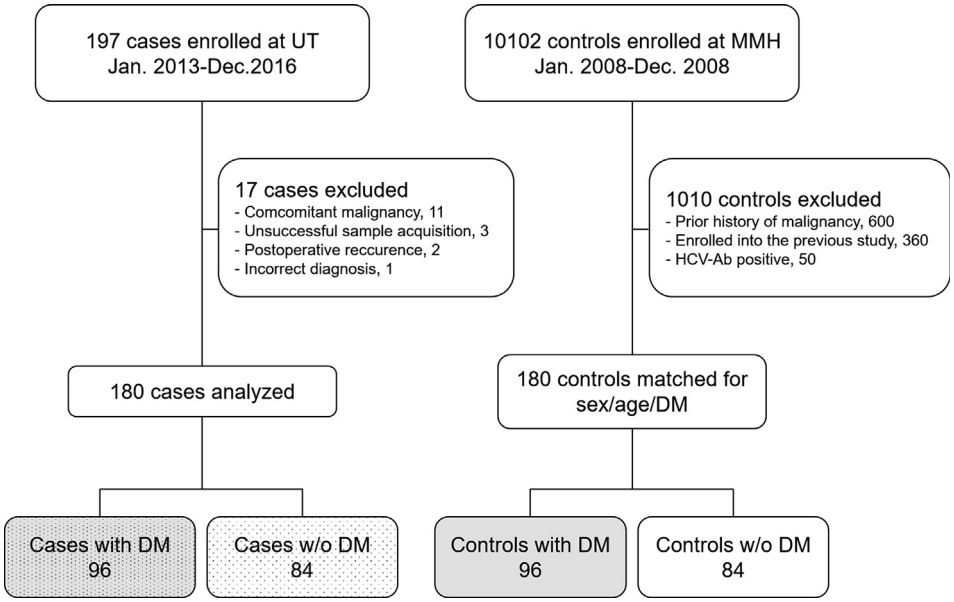

Fig. 1. Flowchart of the study participants. Cases were recruited from the University of Tokyo (UT), and controls were recruited from Mitsui Memorial Hospital (MMH).

Methods

We conducted a matched case–control study to compare the diagnostic yield of the PFAA index for PaC between individuals with and those without DM. Cases with PaC were prospectively recruited from the University of Tokyo between January 2013 and December 2016. The diagnosis of PaC was confirmed pathologically, and the clinical stage was classified according to the Union for International Cancer Control classification. Cases with active concomitant malignancy were excluded. Controls without PaC were prospectively recruited from the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital between January 2008 and December 2008. Controls with a history of malignancy and those enrolled in training set in the previous study [7] were excluded. Cases and controls were all Japanese and ≥20 years of age. Matched controls based on DM status, age, and sex were selected at a 1:1 ratio using the nearest neighbor matching method. DM was defined as undergoing glucose-lowering therapy, HbA1c ≥ 6.5%, and/or fasting blood glucose level ≥126 mg/dl [12]. This study was approved by all institutional review boards, and written informed consent was obtained from all cases and controls.

PFAA concentrations were measured by liquid chromatography–mass spectrometry, and the PFAA index was calculated based on the concentrations of the following six amino acids: Ser, Ile, His, Asn, Ala, and Trp [7]. Scores ≥8.0 were considered positive for the PFAA index, which corresponded to a specificity ≥95% in a previous study [7].

We compared the sensitivity and specificity of the PFAA index for PaC between patients with and those without DM. All statistical analyses were performed using JMP Pro (version 14.0.0; SAS Institute, Cary, NC, USA) or R software (version 3.4.1; The R Foundation for Statistical Computing, Vienna, Austria). Continuous variables are expressed as means (standard deviation) and were compared using the *t*-test. Categorical variables are expressed as numbers (%) and were compared using the chi-square test. We used the Cochran–Armitage trend test to analyze the sensitivity of the PFAA index according to the PaC clinical stage. A *P*-value < 0.05 was considered significant.

Results

Fig. 1 shows a flowchart of the participants selected for this study. Of the 197 cases recruited, 17 were excluded, and thus 180 cases were included in the study; matched controls were selected at a 1:1 ratio. The prevalence of DM in this cohort was 53.3% (96/180). Cases and controls were well matched for sex and age. The cases had a lower body mass index but a higher HbA1c both in subjects with and those without DM, compared with the controls (Table 1). The PaC clinical stage was comparable between cases with and those without DM, but PaC of the head was more prevalent in cases without DM (Table 2).

In this study, the sensitivity and specificity of the PFAA index were 61.7% and 93.3%, respectively. According to the original report on the PFAA index, the sensitivity was 60.0% in the training set and

Table 1
Characteristics of the study participants.

	Cases with DM	Controls with DM	P-value	Cases w/o DM	Controls w/o DM	P-value
N	96	96		84	84	
Sex*, male	61 (63.5)	69 (71.9)	0.22	49 (58.3)	50 (59.5)	0.88
Age [years]	70.0 (8.1)	68.8 (7.1)	0.30	65.3 (9.9)	65.3 (9.9)	0.98
BMI [kg/m^2]	21.8 (3.2)	23.7 (3.4)	<0.01	21.2 (3.5)	22.8 (2.8)	<0.01
HbA1c [%]	7.7 (1.5)	6.6 (0.8)	<0.01	5.7 (0.5)	5.4 (0.3)	<0.01

Values are expressed as means (standard deviation) otherwise indicated.
* Values are expressed as numbers (%).
DM, diabetes mellitus

Table 2
Characteristics of pancreatic cancers.

		Cases with DM	Cases w/o DM	P-value
N		96	84	
Locus of the tumor, head		40 (41.7)	51 (60.7)	0.01
Size of the primary tumor* [mm]		42.0 (22.4)	37.0 (14.9)	0.09
Stage	I	1 (1.0)	2 (2.4)	0.43
	II	24 (25.0)	20 (23.8)	
	III	27 (28.1)	16 (19.1)	
	IV	44 (45.8)	46 (54.8)	

Values are expressed as numbers (%) otherwise indicated.
* Values are expressed as means (standard deviation).
DM, diabetes mellitus.

57.5% in the validation set, with specificity of 95.0% [7].

We explored the association between the diagnostic yield of the PFAA index and DM. The sensitivity of the PFAA index was 66.7% in cases with DM and 56.0% in those without DM ($P = 0.14$), and the specificity was 92.7% in controls with DM and 94.0% in those without DM ($P = 0.95$) (Fig. 2). Therefore, the diagnostic yield of the PFAA index for PaC was not affected by the presence of DM.

The sensitivity of the PFAA index according to the PaC clinical stage is shown in Fig. 3. The sensitivity increased with the more advanced disease stage in cases with and those without DM. The sensitivity in cases with DM was 0% for stage I, 58% for stage II, 56% for stage III, and 80% for stage IV PaC ($P = 0.01$). The sensitivity in cases without DM was 0% for stage I, 35% for stage II, 56% for stage III, and 67% for stage IV PaC ($P < 0.01$).

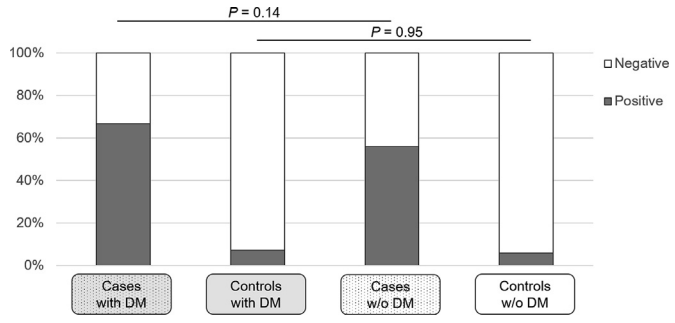

Fig. 2. Positive rate for the plasma free amino acid (PFAA) index. DM, diabetes mellitus.

Discussion

In this matched case–control study, the PaC diagnostic yield of the PFAA index [7] in patients with DM was comparable with that in non-diabetic subjects. The sensitivity and specificity of the PFAA index in patients with DM were 66.7% and 92.7% respectively, which were comparable with those in non-diabetic subjects.

Although screening for PaC is important in high-risk individuals, such as those with multiple relatives with PaC, those with Peutz–Jeghers syndrome, and gene mutation carriers (*BRACA2*, *PALB2*, and *p16*) with affected first-degree relatives [13], screening in a non-selected population is considered inefficient in terms of the cost–benefit balance. Selecting high-risk subjects is essential for a cost-effective PaC screening program.

DM is widely accepted as a risk factor for PaC [8,9]. Hyperglycemia, hyperinsulinemia, and insulin resistance are considered to be associated with carcinogenesis [14]. In addition, DM has recently been considered a symptom of PaC [8]. Among patients with PaC, an impaired glucose tolerance precedes the diagnosis of PaC [15,16], and new-onset DM within 2 years is more prevalent among

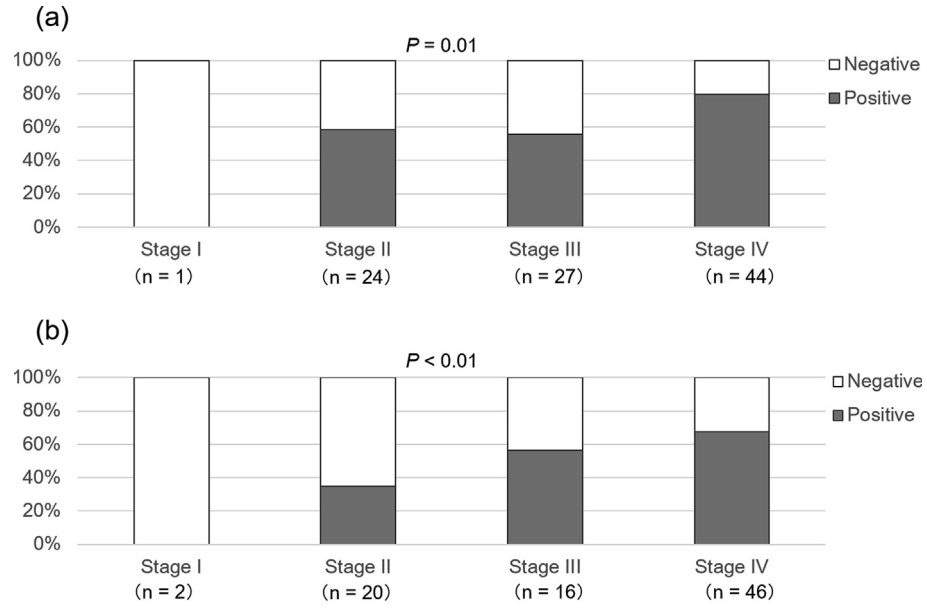

Fig. 3. Sensitivity of the plasma free amino acid (PFAA) index according to the clinical stage of pancreatic cancer. The clinical stage was classified according to the Union for International Cancer Contorol classification. (a) Cases with diabetes mellitus (DM). (b) Cases without DM.

patients with PaC [2,17]. Thus, DM is considered both a risk factor for and a consequence of PaC and is a strong diagnostic candidate for PaC.

A number of biomarkers for diagnosing PaC have been reported in recent studies [3–6]. Among them, metabolomics is an intensively investigated approach [18–22]. Fukutake et al. evaluated the profile of PFAAs in patients with PaC compared with healthy controls and developed the PFAA index, which showed a sensitivity of 57.5% and specificity of 95% [7].

In this study, we applied this PFAA index to patients with DM at high risk of developing PaC because PFAA concentrations are reportedly altered by DM [10,11,23,24]. Actually, plasma concentrations of Ile and phenylalanine are increased in patients with DM, and they were increased significantly in patients with DM in our cohort, suggesting the generalizability of our study results. As our study demonstrated comparable diagnostic performance for the PFAA index between PaC patients with and those without DM, we believe the PFAA index is a “second sieve” [25] to select patients with DM for further invasive investigations regarding PaC, such as endoscopic ultrasonography and computed tomography.

The major limitations of our study were its cross-sectional design and the small sample size. In addition, the low sensitivity of the PFAA index for early-stage PaC may raise concerns over the utility of this for PaC screening. Data on the duration of DM were lacking in the control group, which is important for PaC risk stratification. A meta-analysis of cohort studies involving patients with DM demonstrated a negative correlation between the relative risk of PaC and the duration of DM, with the highest risk of PaC seen within 1 year from DM onset [9]. Given the relatively high risk of PaC in new-onset DM, the role of PFAAs should be further investigated in this subgroup.

In conclusion, we demonstrated the potential utility of the PFAA index for diagnosing PaC in patients with DM.

Conflicts of interest

This was a collaborative research project between the University of Tokyo and Ajinomoto Co., Inc. This study was funded by Ajinomoto Co., Inc., and N.S., T.Tag., S.K., and H.Y. are employees of Ajinomoto, Co., Inc. M.Y. serves as a consultant to Ajinomoto, Co., Inc. and receives lecture fee from Ajinomoto, Co., Inc. The funder provided support in the form of salaries for N.S., T.Tag., and S.K. but did not play any additional role in the study design, data collection or analysis, decision to publish, or preparation of the manuscript.

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