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Urine diacetylspermine as a novel tumour maker for pancreatobiliary carcinomas

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Abstract

Background. Serum carcinoembryonic antigen (highly specific) and carbohydrate antigen 19-9 (highly sensitive) have been used as tumour markers for pancreatobiliary caners. A novel urine tumour marker, diacetylspermine, was compared with the two conventional serum tumour markers in 125 patients with pancreatobiliary diseases.

Results. When the diagnosis of benign or malignant condition was examined, the sensitivity of urine diacetylspermine (75%) was higher than that of serum carcinoembryonic antigen (44%; $P = 0.048$) and the same as that of serum carbohydrate antigen 19-9 (75%). The specificity of urine diacetylspermine (81%) was lower than that of serum CEA (92%) and as high as that of serum carbohydrate antigen 19-9 (80%). The efficiency of urine diacetylspermine (79%) was higher than that of serum carcinoembryonic antigen (74%) and the same as that of serum carbohydrate antigen 19-9 (79%).

Conclusion. These results suggest that urine diacetylspermine is a marker for pancreatobiliary carcinoma, which is as highly sensitive and specific as serum carbohydrate antigen 19-9.

Keywords: Carbohydrate antigen 19-9; Carcinoembryonic antigen; Diacetylspermine

1. Introduction

Polyamines are ubiquitous organic polycations that are synthesised in substantial amounts by rapidly growing and dividing cells, including cancer cells [1]. Polyamines excreted in the urine are mainly in the monoacetylated form. Among them, acetylputrescine is most abundant followed by acetylcadaverine, N^1-acetylspemidine and N^8-acetylspermine. Diacetylated forms are minor components and the average amounts of diacetylspermidine and diacetylspermine are only 1.4 and 0.46%, respectively [2]. These diacetylated polyamine species have not been identified because they are of limited quantity in the urine and are undetectable by conventional methods due to a lack of the primary amino groups.

Russell [3] first reported increased excretion of polyamines in the urine in cancer patients, which evoked a surge of studies on polyamine analysis in the urine in 1970s [1]. However, the following results were disappointing because there were too many false negative and false positive results as far as total polyamine levels and its monoacetyl compounds were examined [4], although their average urinary levels were certainly higher in patients with cancer than in healthy persons [4,5]. Therefore, diagnostic utility of urine polyamines has been abandoned for a long time.

Recently, Hiramatsu et al. [2,6,7] reported that diacetylspermine, one of spermine derivatives, is more frequently expressed in the urine of patients with malignant conditions compared to benign diseases using high-performance liquid

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chromatography (HPLC) procedure. However, HPLC is difficult, not so popular and cannot be used as a clinical laboratory test. Thereafter, Hiramatsu et al. [8] developed a sensitive and accurate enzyme-linked immunosorbent assay (ELISA) system with the use of an affinity-purified polyclonal antibody against diacetylspermine. ELISA is easy to use, and therefore, appropriate to be used in the clinical laboratory.

Serum carcinoembryonic antigen (CEA) [9,10] and carbohydrate antigen (CA) 19-9 [11] have been used as tumour markers for pancreatobiliary caners. Serum CEA is highly specific for pancreatobiliary cancers and serum CA19-9 is highly sensitive. Their positivity increases with the progression of the diseases. They are not useful in detecting early cancers but are used as a marker of the recurrence of the tumour after surgical resection.

There are few reports of urine diacetylspermine levels in patients with pancreatobiliary diseases. In this series, clinical implications of this novel urine tumour marker, diacetylspermine, were examined by comparing with two conventional tumour markers, serum CEA and CA19-9, in 125 patients with pancreatobiliary diseases.

2. Materials and methods

This series consisted of 125 patients with pancreatobiliary diseases who were treated in the Department of Surgery I, Kyushu University Hospital, Fukuoka, Japan from November 2002 to January 2003. The 125 patients were 70 men and 55 women and their age ranged from 28 to 86 years with a mean of 63.5 ± 12.2 years. The 125 patients included 52 patients in the preoperative or postoperative state of benign diseases (control group), 22 patients in the preoperative state of malignant diseases, and the other 51 patients in the postoperative state (more than 3 months) of malignant conditions. Of the 51 postoperative patients, 10 had unequivocal signs of recurrence as judged by the clinical findings including imaging and the others had no definite evidence of recurrence as proven by subsequent clinical follow-up as well. Therefore, a total of 32 patients were considered to have malignant pancreatobiliary tumours. The 52 patients in the control group consisted of 28 with benign inflammatory diseases [15 in the preoperative state (9 chronic pancreatitis, 3 hepatolithiasis, 1 cholecystolithiasis, 2 adenomyomatosis of the gallbladder) and 13 in the postoperative state (10 chronic pancreatitis, 1 cholecystolithiasis, 1 choledocholithiasis and 1 choledochal cyst)] and 24 with adenoma (8 in the preoperative state of intraductal papillary-mucinous adenoma of the pancreas, 15 in the postoperative state of the same disease, 1 in the postoperative state of adenoma of the papilla of Vater). Urine diacetylspermine is known to be elevated in acute inflammatory conditions including acute pancreatitis, acute cholecystitis and the early postoperative state due to the rapid turnover of cells (personal communication with Dr. M. Kawakita). All patients who suffered from acute inflammatory diseases and were within 3 months after the operation were excluded from the present series. The patients with pancreatic endocrine tumours and hepatocellular carcinomas were also excluded because we intended to compare urine diacetylspermine and serum CEA and CA19-9. Urine diacetylspermine and serum CEA and CA19-9 were examined in all these 125 patients.

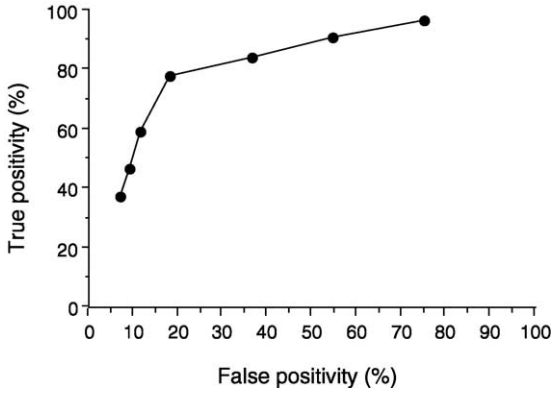

Fig. 1. Receiver operating characteristic curve indicating test performance for pancreatobiliary carcinoma for the following threshold values: 175, 225, 275, 325, 375, 425 and 475 nmol/g Creatinine. (●–●) Performance in distinguishing malignant from benign pancreatobiliary diseases.

Urine and peripheral blood were obtained in the morning. Diacetylspermine was measured by using ELISA system, which was created by Transgenic Co. Ltd, Kumamoto, Japan according to the Hiramatsu's method [8] in CRC Co. Ltd., Fukuoka, Japan. The cut-off level was set at 325 nmol/g Creatinine by constructing a receiver operating characteristics curve (Fig. 1). The serum levels of CEA and CA19-9 were measured in the Clinical Laboratory of Kyushu University Hospital. Their cut-off levels were 2.5 ng/ml and 37.4 IU/ml, respectively.

The clinical stage of malignant pancreatobiliary diseases was determined according to TNM Classification of Malignant Tumours issued by the UICC [12]. The 32 patients with primary or metastatic pancreatobiliary adenocarcinoma consisted of six in Stage IIb, two in Stage III and 24 in Stage IV.

Informed consent was obtained from each patient. The protocol was submitted to and preapproved by the Senior Staff Committee of the Department.

Values were expressed as mean ± standard deviation. Mean values were compared by Student's *t* test and the distribution of patients was measured by the chi-square test. The sensitivity, specificity, positive predictive value, negative predictive value and efficiency were measured. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Measurements of urine diacetylspermine

Urine diacetylspermine levels of the 52 patients in the control group and 32 with pancreatobiliary adenocarcinoma were 267.2 ± 143.0 and 621.5 ± 584.0 nmol/g Creatinine,

Table 1
Malignant and benign diagnosis by urinary diacetylspermine, serum CEA and CA19-9

	Diacetylspermine		CEA		CA19-9	
	Positive	Negative	Positive	Negative	Positive	Negative
Malignant	22	10	14	18	21	11
Benign	8	44	4	48	7	45
Sensitivity (%)	75[a]		44[a]		75	
Specificity (%)	81		92		80	
Positive predictive value (%)	71		78		86	
Negative predictive value (%)	84		73		87	
Efficiency (%)	79		74		79	

[a] $P = 0.044$.

respectively ($P < 0.001$). The serum CEA and CA19-9 values of the two groups were 1.2 ± 0.8 and 39.2 ± 158.5 ng/ml in the controls and 16.8 ± 21.3 and $7{,}825.2 \pm 23{,}996.8$ U/ml in the malignant group ($P = 0.0209$), respectively. The values of eight patients with intraductal papillary-mucinous adenoma were 243.2 ± 96.1 nmol/g Creatinine, 1.4 ± 1.2 ng/ml and 9.0 ± 5.0 U/ml, which were similar to those of the control group and were lower than those in patients with adenocarcinoma, but the differences were not statistically significant.

3.2. Diagnosis of benign and malignant conditions

Data concerning benign and malignant conditions are shown in Table 1. The sensitivity of urine diacetylspermine for malignant conditions was 75%, which was higher than 44% ($P = 0.044$) of serum CEA and as high as 75% of serum CA19-9. The specificity of urine diacetylspermine was 81%, which was lower than 92% of serum CEA and as high as 82% of serum CA19-9. Efficiency of urine diacetylspermine was 79%, which was similar to 74% of serum CEA and 79% of serum CA19-9.

3.3. Detection of recurrence of pancreatobiliary carcinoma

Data are present in Table 2. The sensitivity of urine diacetylspermine for the presence of recurrence of malignant pancreatobiliary diseases was 80%, which was higher than 70% of serum CEA and 70% of serum CA19-9. The specificity of urine diacetylspermine was 66%, which was lower than 78% of CEA and 83% of CA19-9.

Table 3
Tumour stage and urine diacetylspermine, serum CEA and CA19-9

Stage	Diacetylspermine		CEA		CA19-9	
	Positive	Negative	Positive	Negative	Positive	Negative
IIb	3	3	0	6	4	2
III	2	0	1	1	1	1
IV	19	5	11	13	16	8

3.4. Tumour stage and three markers

The mean values of urine diacetylspermine of patients with Stages IIb, III and IV carcinoma were 347.8 ± 139.7, 605.1 ± 375.9, 691.3 ± 651.7 nmol/g Creatinine, serum CEA 0.9 ± 0.1, 15.6 ± 20.9 and 50.8 ± 182.4 ng/ml and serum CA19-9 134.2 ± 154.6, 1726.1 ± 2438.2 and $10{,}256.3 \pm 27{,}405.1$ U/ml, respectively. The positivity of the three markers increased with the progression of tumours (Table 3). Three of the six patients with Stage IIb carcinoma showed a positive urine diacetylspermine result. None of them showed elevation of serum CEA, but four of them did show elevation of serum CA19-9.

3.5. Predictivity for unresectability of carcinoma by three markers

The sensitivity for prediction of unresectability of urine diacetylspermine (81%) was higher than that of serum CEA

Table 2
Recurrence and urinary diacetylspermine, serum CEA and CA19-9

	Diacetylspermine		CEA		CA19-9	
	Positive	Negative	Positive	Negative	Positive	Negative
Recurrence						
Yes	8	2	7	3	7	3
No	14	27	9	32	7	34
Sensitivity (%)	80		70		70	
Specificity (%)	66		78		83	
Positive predictive value (%)	36		44		50	
Negative predictive value (%)	93		91		92	
Efficiency (%)	69		76		80	

Table 4
Unresectability and three markers

	Diacetylspermine		CEA		CA19-9	
	Positive	Negative	Positive	Negative	Positive	Negative
Unresectability						
Yes	21	5	14	12	17	9
No	3	3	0	6	4	2
Sensitivity (%)	81[a]		54[a]		65	
Specificity (%)	50		100		33	
Positive predictive value (%)	88		100		81	
Negative predictive value (%)	38		33		18	
Efficiency (%)	75		63		59	

[a] $P = 0.039$.

(54%, $P = 0.039$) and serum CA19-9 (65%) (Table 4). The specificity of serum CEA was highest of the three markers. The efficiency of urine diacetylspermine (75%) was higher than that of serum CEA (63%) and serum CA19-9 (59%), but the differences were not significant.

4. Discussion

Serum levels of CA19-9 (highly sensitive) and CEA (highly specific) have been used as tumour markers for pancreatobiliary diseases. Usefulness of urine diacetylspermine as a tumour marker was studied in 125 patients with pancreatobiliary diseases. The sensitivity of urine diacetylspermine was higher than that of serum CEA and as high as that of CA19-9 and the specificity of urine diacetylspermine was also as high as those of serum CEA and CA19-9. A half of the patients with Stage IIb pancreatobiliary carcinoma gave a positive result for urine diacetylspermine. Urine diacetylspermine is a novel tumour marker for pancreatobiliary carcinoma, being as highly sensitive and specific as serum CA19-9.

It is true that ideal tumour markers should have high specificity and sensitivity for target malignant conditions. This urine diacetylspermine showed sensitivity and specificity similar to serum CA19-9 and CEA, respectively. Because the urine sample is easy to obtain and the measurement could be done by the ELISA kit, urine diacetylspermine is a novel convenient tumour marker for pancreatobiliary carcinomas.

The clinical outcome of patients with pancreatobiliary carcinoma remains dismal despite the recent progress of diagnostic and therapeutic modalities. Therefore, early diagnosis of pancreatobiliary carcinoma is mandatory. Conventional tumour markers such as serum CA19-9 and CEA have been reported to be not useful in detecting early pancreatic carcinoma. Urine diacetylspermine was positive in a half of patients with Stage IIb pancreatobiliary carcinoma, although the total number of patients examined was small. Therefore, this marker may be of value to detect early pancreatobiliary cancer.

Clinical differentiation between benign inflammatory diseases and malignant diseases is important as well as that of benign and malignant neoplasms. The urine diacetylspermine level is increased in the conditions where cell turnover is accelerated. Thus, patients in the phase of acute inflammation and in the early postoperative state were excluded from the present series. Urine diacetylspermine in patients with benign pancreatobiliary neoplasms were as low as that in those with chronic inflammatory pancreatobiliary diseases, and both the levels were lower than that of pancreatobiliary carcinoma. Therefore, urine diacetylspermine seems to be useful to distinguish malignant pancreatobiliary diseases from benign ones.

It is true that the surgical decision is made by imaging and/or macroscopic findings, but the prediction of resectability by serum tumour markers, if possible, is of great value for surgeons. The positivity and values of urine diacetylspermine increased with the progression of the malignant diseases. Therefore, the urine diacetylspermine level may be of some value to predict the choice of the treatment.

One of the other purposes of tumour marker determination is to predict the clinical outcome of patients with pancreatobiliary carcinoma. This series is a preliminary report on urine diacetylspermine in patients with pancreatobiliary diseases and there is no long-term information. Therefore, further examination is mandatory to identify the usefulness of this novel maker as a prognostic indicator.

Theoretically, urine diacetylspermine is considered to be not specific for a special type of malignancy but for all malignant conditions of any organs. Sugimoto et al. [6] reported that urine diacetylpolyamine was markedly increased in patients with urogenital malignancies and van den Berg et al. [13] reported a high concentration of diacetylspermine in the urine of patients with non-Hodgkin's lymphoma by capillary gas chromatography. In this series, a high concentration of urine diacetylspermine was proved in patients with pancreatobiliary carcinoma. Concerning pancreatic cancer, we previously reported a high incidence of pancreatic cancer in selected patients with diabetes mellitus [14] and intraductal papillary-mucinous neoplasm of the pancreas [15]. This novel marker may be of great value in mass screening and follow-up of patients at high risks of the development of malignancy such as familial adenomatosis coli, ulcerative coli-

tis, pancreatobiliary malunion, congenital choledochal cyst, diabetes mellitus, intraductal papillary-mucinous tumour of the pancreas [16], Barret's oesophagus and so on.

Although this is a preliminary report on urine diacetylspermine in patients with pancreatobiliary diseases, this novel urinary marker is as sensitive and specific for pancreatobiliary carcinomas as serum CA19-9. Urine diacetylspermine may be a universal tumour marker for malignant conditions and useful in mass screening and follow-up of high-risk groups.

Conflict of interest statement

None declared.

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