The clinical usefulness of urinary N^1,N^{12}-diacetylspermine (DiAcSpm) levels as a tumor marker in patients with colorectal cancer

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Abstract. The aim of this study was to evaluate the usefulness of urinary N^1,N^{12}-diacetylspermine (DiAcSpm) measured by the colloidal gold aggregation method as a tumor marker for colorectal cancer (CRC). The preoperative urine of 113 CRC patients was collected, and the urinary DiAcSpm was measured by a reagent kit for DiAcSpm determination based on colloidal gold aggregation using automatic biochemical analyzers. The urinary DiAcSpm levels significantly correlated with distant metastasis and Tumor-Node-Metastasis (TNM) stage. The positive rates of urinary DiAcSpm were significantly higher than those of serum carcinoembryonic antigen (CEA) or cancer antigen 19-9 (CA19-9) in stages 0+I, II, III and IV. The positive rates of urinary DiAcSpm were also significantly higher than those of serum CEA or CA19-9 in the early and advanced CRC groups according to the Japan Classification of Colorectal Cancer. Therefore, urinary DiAcSpm, measured by a reagent kit for DiAcSpm determination based on colloidal gold aggregation, may be useful as a non-invasive tumor marker in patients with CRC.

Introduction

Colorectal cancer (CRC) is one of the most fatal types of cancer worldwide. In Japan, CRC was estimated to be the most common cause of female cancer-related mortality and the third most common cause of male cancer-related mortality in 2006 (1). Although chemotherapy has had a beneficial impact on the overall survival of patients with metastatic CRC, the prognosis remains relatively poor. Therefore, the identification of tumor markers that could detect early CRC and determine CRC patients at a high risk of recurrence are required.

Polyamines, including putrescine, cadaverine, spermidine and spermine, are alkylamines with multiple amino groups that are ubiquitously produced by eukaryotic and prokaryotic cells. Their metabolites are acetylated and diacetylated derivatives, respectively. Rapidly growing tissues usually have active polyamine synthesizing systems and contain large amounts of polyamines (2). Polyamine excretion in the urine may increase when such tissues are present in the body. A previous study indicated that the amount of polyamines excreted in the urine was higher in patients with cancer compared to that in healthy individuals (3). However, results of subsequent studies showed that the total amount, as well as the amount of individual free and monoacetylated polyamines in the urine, were not suitable as reliable tumor markers, as these yielded a number of false positive and negative results (4,5).

N^1,N^{12}-diacetylspermine (DiAcSpm) is a minor component of human urine that constitutes less than 0.5% of the total polyamine species in normal human urine. DiAcSpm was initially detected and characterized by high-performance liquid chromatography (HPLC) fractionation (6,7), followed by enzymatic detection. Antibodies highly-specific for DiAcSpm were previously prepared, and an enzyme-linked immunosorbent assay (ELISA) system applicable for the determination of urinary DiAcSpm was established (8,9). Several studies have reported that DiAcSpm in the urine measured by ELISA may be useful as a novel tumor marker of CRC (10,11), breast cancer (10,11) and hepatocellular carcinoma (12). Since then, the development of an ELISA procedure for measuring the DiAcSpm in the urine has enabled extensive clinical studies to be conducted.

A reagent kit for DiAcSpm determination based on colloidal gold aggregation that can be used with automatic biochemical analyzers was recently developed (13,14). This method is considered to be more rapid and more convenient than the ELISA method. Therefore, we evaluated the usefulness of urinary DiAcSpm as a tumor marker for CRC using a reagent kit for DiAcSpm determination based on colloidal gold aggregation.

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Patients and methods

Patients. A total of 113 patients with primary CRC who underwent surgery at the Department of Surgery at the Wakamatsu Hospital of the University of Occupational and Environmental Health, Fukuoma, Japan, from 2008 to 2010 were recruited for this study, and their clinical data were analyzed (Table I). Of the 113 patients, 90 patients underwent curative surgery, and 23 patients underwent palliative surgery, including palliative resection, construction of an artificial anus or implantation of a central venous feeding tube and port for chemotherapy. Informed consent was obtained from all patients prior to the study. No patients received chemotherapy or radiotherapy prior to surgery. The clinicopathological findings were determined according to the Union for International Cancer Control (UICC) Tumor-Node-Metastasis (TNM) classification (15).

Urine samples. Urine samples were collected from all 113 patients with CRC after informed consent was obtained. These samples were frozen at -20˚C until use.

Determination of DiAcSpm by colloidal gold aggregation. The urinary DiAcSpm concentrations were determined using an Auto DiAcSpm reagent kit (Alfresa Pharma Co., Osaka, Japan) at the Alfresa Pharma research assay laboratory, according to the manufacturer's instructions. The assay is based on the specific binding between a bovine serum albumin-acetylspermin conjugate, as a DiAcSpm mimic, and a stable red-purple solution of colloidal gold antibody complexes (13). The cut-off values for urinary DiAcSpm, which were the mean +2 SD for healthy subjects, were 210 nmol/g•Cre in males and 250 nmol/g•Cre in females, respectively.

Clinicopathological assessment. The tumors were staged by two pathologists, who had no prior knowledge of the results of the assays, according to the 7th edition of the UICC TNM classification (15). Clinicopathological factors, including age, gender, tumor size, histological type, depth of invasion, lymph node metastasis, distant metastasis and staging, were analyzed for associations with the urinary DiAcSpm levels.

Statistical analysis. Data are presented as the mean ± SD and were statistically analyzed using the Student's t-test and regression analysis, as appropriate. The correlations between the parameters were also assessed statistically using the χ^2 test with the Stat View-J statistical software package (version 5.0, SAS Institute, Inc., Cary, NC, USA).

Results

A total of 113 patients diagnosed with primary CRC and their characteristics were analyzed (Table I). The urinary DiAcSpm levels in the patients with stage IV disease were significantly elevated compared with those in the patients with stage 0+I, II or III disease (Table II). Similarly, the serum CEA levels in the patients with stage IV disease were significantly elevated compared with those in patients with stage 0+I or II disease (Table II). However, the serum CA19-9 levels were not significantly different among the patients with different disease stages (Table II).

Table I. Characteristics of the 130 patients with colorectal cancer.

Characteristic	No.
No. of patients	113
Gender	
Male	64
Female	49
Age (years) (mean ± SD)	69.3±11.0
Location	
Colon	76
Rectum	37
Histological type	
Differentiated	106
Undifferentiated	7
Depth of invasion	
Tis (0)	1
T1	8
T2	18
T3	53
T4a	22
T4b	11
Metastasis	
Lymph node (-/+)	52/61
Distant (-/+)	90/23
TNM stage	
0	1
I	18
II	32
III	39
IV	23

TNM, Tumor-Node-Metastasis. SD, standard deviation.

The urinary DiAcSpm levels significantly correlated with the presence of distant metastasis and the TNM stage. However, the DiAcSpm levels did not correlate with clinicopathological characteristics, such as patient age, gender, histopathological type, depth of invasion or presence of lymph node metastasis (Table III).

According to the evaluation of the urinary DiAcSpm levels, the positive rates for the urinary DiAcSpm were 66.7 in stage 0+I patients, 71.9 in stage II, 48.7 in stage III and 100% in stage IV patients, respectively, which demonstrated a total rate of 69.0% in 113 patients. The positive rates of the serum CEA were 27.8 in patients with stage 0+I disease, 46.9 in stage II, 38.5 in stage III and 78.3% in stage IV, respectively, demonstrating a total rate of 44.2% in all patients. The positive rates of serum CA19-9 were 22.2 in those of stage 0+I disease, 15.7 in stage II, 20.5 in stage III and 56.5% in stage IV, respectively, which demonstrated a total rate of 26.5% in all 113 patients (Table IV). The positive rates of urinary DiAcSpm

Table II. Preoperative levels of urinary DiAcSpm, serum CEA and CA19-9 in patients with colorectal cancer.

TNM stage	No. of cases	Urinary DiAcSpm (nmol/g•Cre)	Serum CEA (ng/ml)	Serum CA19-9 (U/ml)
0+I	19	258.6±101.1	2.0±1.2	60.3±194.9
II	32	625.8±1204.3	6.1±11.1	19.7±20.3
III	39	367.9±548.0	14.2±54.1	25.2±45.0
IV	23	1935.7±2412.9[a]	45.2±79.6[a]	1290.4±4850.2
Total	113	742.0±1426.0	16.2±50.1	287.1±2210.8

The urinary DiAcSpm levels in patients with stage IV disease were significantly elevated compared with those in patients with stage 0+I, II or III disease. Similarly, the serum CEA levels in patients with stage IV disease were significantly elevated compared with those in the patients with stage 0+I or II disease. [a]$p< 0.05$. DiAcSpm, N^1,N^{12}-diacetylspermine; CEA, carcinoembryonic antigen; CA19-9, cancer antigen 19-9; TNM, Tumor-Node-Metastasis.

Table III. Association between the preoperative levels of DiAcSpm and clinicopathological factors of the patients with colorectal cancer.

	Preoperative value of DiAcSpm[a]		
Variables	Negative	Positive	P-value
Age (years)			
≤69.3	15	48	
>69.3	19	31	0.1023
Gender			
Male	19	45	
Female	15	34	0.9154
Location			
Colon	22	54	
Rectum	12	25	0.7046
Histological type			
Differentiated	31	75	
Undifferentiated	3	4	0.4469
Depth of invasion (T)			
≤T2	8	19	
T3≤	26	60	0.7203
Lymph node metastasis (N)			
(-)	15	37	
(+)	19	42	0.7904
Distant metastasis (M)			
(-)	34	36	
(+)	0	23	0.0004
TNM stage			
I/II/III	34	36	
IV	0	23	0.0004

[a]The cut-off values for urinary DiAcSpm, which represent the mean+ 2 SD for healthy subjects, were 210 nmol/g•cre in males and 250 nmol/g•cre in females, respectively. DiAcSpm, N^1,N^{12}-diacetylspermine.

were significantly higher than those of serum CEA or CA19-9 in patients with all stages of CRC (Table IV).

The patients were divided into an early and an advanced CRC group according to the Japan Classification of Colorectal Cancer. The positive rates in the early CRC group were 66.7 for DiAcSpm, 11.1 for CEA and 11.1% for CA19-9, and the positive rates in patients with advanced CRC were 69.2 for DiAcSpm, 47.1 for CEA and 27.9% for CA19-9, respectively. The positive rates of urinary DiAcSpm in the early and advanced CRC groups were significantly higher than those of serum CEA or CA19-9 (Table V).

Discussion

The present study has shown the usefulness of urinary DiAcSpm as a tumor marker for CRC as measured by the colloidal gold aggregation method. This method is considered to be more rapid and more convenient compared with the ELISA system, as this method uses an Auto DiAcSpm reagent kit method on colloidal gold aggregation and an automatic clinical analyzer. This method is able to measure a large number of samples at one time compared with the ELISA method.

Certain studies have reported the usefulness of the urinary DiAcSpm level as measured by ELISA in patients with CRC (10,11), breast cancer (10,11), and hepatocellular carcinoma (12). The positive rate of urinary DiAcSpm measured by ELISA for CRC patients (n=248) was 75.8%, which was markedly higher than the positive rates for serum CEA (39.5%, $p<0.0001$) and CA19-9 (14.1%, $p< 0.0001$) (10). Another study indicated that the sensitivity of DiAcSpm measured by ELISA in CRC patients (n=33) was 69.6%, which was also superior to that of serum CEA (46.8%) and CA19-9 (15.6%) (11). Hiramatsu *et al* also reported that the positive rate of urinary DiAcSpm measured by ELISA for 83 cases of breast cancer (60.2%) was higher than the sensitivities of CEA (37.3%, $p=0.0032$) and CA15-3 (37.3%, $p=0.0032$) (10). A further study indicated that the sensitivity of DiAcSpm measured by ELISA in breast cancer patients (n=28) was 46.4%, which was higher than that of serum CEA (3.8%) and CA15-3 (0%) (11). These studies have demonstrated that the urinary DiAcSpm level, as measured by ELISA, is useful as a tumor marker in patients with CRC and breast cancer. However, the positive

Table IV. Preoperative positive ratio (%) of urinary DiAcSpm, serum CEA and CA19-9 in patients with colorectal cancer.

TNM stage	No. of cases	Urinary DiAcSpm (%)	Serum CEA (%)	Serum CA19-9 (%)
0+I	19	66.7[a]	27.8	22.2
II	32	71.9[b]	46.9	15.7
III	39	48.7[a]	38.5	20.5
IV	23	100.0[a]	78.3	56.5
Total	113	69.0[b]	44.2	26.5

The positive rates of urinary DiAcSpm were significantly higher than those of serum CEA or CA19-9 in patients with all stages of CRC. [a]$p<0.05$, [b]$p< 0.001$. DiAcSpm, N^1,N^{12}-diacetylspermine; CEA, carcinoembryonic antigen; CA19-9, cancer antigen 19-9; TNM, Tumor-Node-Metastasis.

Table V. Preoperative positive ratio (%) of urinary DiAcSpm, serum CEA and CA19-9 in patients with colorectal cancer.

Status of CRC	No. of cases	Urinary DiAcSpm (%)	Serum CEA(%)	Serum CA19-9 (%)
Early	9	66.7[a]	11.1	11.1
Advanced	104	69.2[b]	47.1	27.9
Total	113	69.0[b]	44.2	26.5

[a]$p< 0.05$, [b]$p< 0.001$. Early; early CRC indicated until submucosal invasion; advanced, advanced CRC indicated more deeply invaded beyond proper muscle by the Japan Classification of Colorectal Cancer. DiAcSpm, N^1,N^{12}-diacetylspermine; CEA, carcinoembryonic antigen; CA19- 9, cancer antigen 19-9; CRC, colorectal cancer.

rate of urinary DiAcSpm as measured by ELISA for hepatocellular carcinoma was 65.5%, which was similar to that for AFP or PIVKA-II (12). Results of the present study indicated that the positive rate of urinary DiAcSpm measured by the Auto DiAcSpm reagent kit based on colloidal gold aggregation in all CRC patients was 69.0%; this positive rate was similar to that measured by ELISA in the other studies (10,11).

In a previous study, the urinary DiAcSpm concentration, as measured by ELISA, was elevated in 60% of TNM colon cancer stage 0+I patients, whereas only 10% ($p<0.0001$) and 5% ($p<0.0001$) of these patients were CEA- and CA19-9-positive, respectively (10). The positive rate of urinary DiAcSpm measured by the colloidal gold aggregation method in the early CRC group was 66.7%, whereas only 11.1 ($p<0.005$) and 11.1% ($p<0.005$) of these patients were CEA- and CA19-9-positive, respectively (Table V). These studies indicated that the urinary DiAcSpm level is elevated from an early stage of CRC, and this increase can be detected by the ELISA and colloidal gold aggregation methods. Therefore, the measurement of urinary DiAcSpm may be suitable for screening patients for CRC. However, although the sensitivity of DiAcSpm for early stage breast cancer (stage I and II; 28.1%) was significantly higher than that of CEA (3.1%; $p=0.0064$) and CA15-3 (0%; $p=0.001$) (10), the positive rate of urinary DiAcSpm in the early stage breast cancer patients was low, therefore the measurement of urinary DiAcSpm may be not suitable for screening patients for breast cancer.

Although the use of conventional tumor markers, including serum CEA or CA19-9, require the relatively invasive collection of blood samples, the collection of urine is easy and does not result in any pain for the patients. Findings of the present study have shown that the urinary DiAcSpm level may be useful as a non-invasive tumor marker measured by a reagent kit for DiAcSpm determination based on the colloidal gold aggregation.

In this study, the positive rate of urinary DiAcSpm measured by the colloidal gold aggregation method in the patients with stage III was lower than the rates of the other stages. However, results from other studies have indicated that the positive rate of the urinary DiAcSpm measured by ELISA in patients with stage III was almost identical to that in the other stages (10). This discrepancy may be due to the small number of patients studied compared with the previous studies. Therefore, continued experiments with a larger number of subjects may be required to confirm the results.

In conclusion, the present study indicated that the urinary DiAcSpm level, as measured by a reagent kit for DiAcSpm determination based on the colloidal gold aggregation, may be useful as a non-invasive tumor marker for detecting patients with CRC.

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