

Urine diacetylated spermine might be a nonspecific tumor marker in canine neoplasia

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Abstract

Diacetylated spermine, a polyamine, is clinically used as a tumor marker in human medicine. The purpose of this study was to evaluate the usefulness of diacetylated spermine as a nonspecific tumor marker in dogs. One hundred six dogs were randomly selected and divided into 4 groups (cancer-bearing, n=23; control, n=44; young, n=24; inflammatory, n=15). Urine samples were collected to measure diacetylated spermine and creatinine levels. The diacetylated spermine concentration was determined by enzyme-linked immunoassay, standardized by creatinine concentration. The urine diacetylated spermine concentration was markedly significant elevated in cancer-bearing dogs, as compared to that in control dogs (P=0.0081). Whilst the urine diacetylated spermine level in cancer-bearing group was not markedly different from that in the young group, there was significant difference between levels in the control and young groups (P=0.019). No significant difference was observed between the inflammatory and cancer-bearing groups. It is suggested that urine diacetylated spermine is a useful tumor marker in dogs over 2 years of age.

Introduction

Presently, the diagnosis and examination of canine cancer patients involves procedures such as histopathological examination, ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). However, expense and/or systemic anesthesia for diagnostic imaging are unfavorable factors. Recently, diagnosis using tumor markers has become topical issue in veterinary medicine. Early detection of cancer is possible, if tumor markers are clinically available; however, there are limited reports about the usefulness of tumor markers in dogs.^{1,2} Some researchers in

the veterinary field have studied tumor markers used in human medicine.

In 1971, the excess urinary excretion of polyamines was reported in human cancer patients.³ Polyamines are classified according to various structures,⁴ and diacetylated spermine, a modified polyamine, is reported to have excellent characteristics as a tumor marker.⁵ Polyamines are polyvalent cations, and affect protein synthesis and nucleic acid synthesis by interacting with nucleic acid or other anions.^{6,7} The physiological function of polyamines is not totally understood; however, polyamines are abundant and metabolically active in actively proliferating tissue.⁵ Thus, polyamines might have important roles in the cellular proliferative pathway. A significant elevation of urine diacetylated spermine was reported in urogenital cancer patients.⁸ Furthermore, urine diacetylated spermine concentration was maintained in the low range during the remission period in human cancer patients; however, elevated levels were detected at the reoccurrence period. Therefore, diacetylated spermine might be useful for monitoring response to treatment, and/or reoccurrence.⁵

In the veterinary field, although some researchers have investigated the association with other tumor markers,¹ urine spermidine and spermine, both polyamines, were elevated in dogs with mammary gland tumors.² Consequently, many investigators suggest that diacetylated spermine might be useful as a nonspecific tumor marker and/or early detection marker of tumor reoccurrence, because of the elevations in human cancer patients. The purpose of this study was to evaluate the usefulness of diacetylated spermine as a nonspecific tumor marker in dogs.

Materials and Methods

Animals

In the present study, 106 dogs were randomly selected and investigated in clinical practice. Urine concentration of diacetylated spermine might be elevated in growing and non-cancer patients (such as those with inflammatory disease). For this reason, animals were divided into 4 groups as follows: cancer-bearing group (n=23), control group (n=44), young group (n=24) and inflammatory diseases group (n=15). Clinical diagnosis of cancer bearing group and inflammatory diseases group are shown in Tables 1 and 2, respectively. The characteristics of each group are summarized in Table 3.

Sample collection

Urine samples were collected by cystocente-

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sis or voiding. For cancer-bearing group, urine samples were collected before treatment and/or during the period of no response to treatment. After screening test, all samples were cryopreserved (-20°C) for a week.

Measurement

Screening test

All collected urine samples underwent sediment and evaluated for WBC, nitrite, urobilinogen, protein, pH, occult blood, specific gravity, keton bodies, bilirubin and glucose (CLINITEL Status @Analyzer; Siemens Co., Ltd., Shinagawa, Tokyo, Japan).

Inflammatory diseases

Inflammatory diseases were based on the elevation of C-reactive protein (CRP, Laser CRP-2; Arrows Co., Ltd., Ibaraki, Osaka, Japan) and/or leukocyte count (XT-1800i; Sysmex Co., Ltd., Kobe, Hyogo, Japan).

Urine diacetylated spermine and urine creatinine concentration

Urine samples were performed by enzyme-linked immunoassay (Metaboscreen Co., Ltd., Yokohama, Kanagawa, Japan) with a commercially manufactured antibody (Mikuri immuno Laboratory Inc., Tanabe, Kyoto, Japan). Urine creatinine concentrations were measured using a Dimension X-pand Plus (Dade Behring Co., Ltd., Shinagawa, Tokyo, Japan). Because of physiological factors and/or circumstances, urine diacetylated spermine concentrations were modified by urine creatinine concentration as followed:

$$\frac{[\text{Diacetylated spermine U}]}{[\text{Creatinine U}]} \times 100$$

Several urine samples including diacetylat-

ed spermine were prepared from cancer dogs to validate our ELISA system; these samples were also evaluated by the commercial available ELISA system (Yamasa 80015; Yamasa

Corporation, Choshi, Chiba, Japan). These two systems did not differ significantly in sensitivity and accuracy within 1 to 64 nM diacetylated spermine. Blood contamination of urine samples acquired using general clinical methods does not affect these systems, and the urine was not treated to remove any blood components in this study.

Table 1. Diagnosed by histopathological examination and/or computed tomography.

Cancer-bearing group (n=23)	N
Lymphosarcoma	3
Malignant mammary gland	5
Mast cell tumor	1
Malignant melanoma	1
Hepatocarcinoma	1
Chronic lymphocytic leukemia	1
Malignant nerve sheath tumor	3
Osteosarcoma	1
Transitional cell carcinoma	1
Malignant mesenchymal sarcoma	1
Brain neoplasia	1
Oral neoplasia	2
Unknown malignant tumor	2

Table 2. Diagnosed by clinical examination and laboratory analysis.

	N
Severe external otitis	7
Arthritis	4
Cystitis	3
Gingivitis	1

Table 3. Averaged age.

	N	Sex ratio (M:F)	Age
Control	44	22:22	6.95±0.52
Inflammation	15	6:9	5.73±0.90
Young	24	12:12	1.44±0.14
Cancer	23	8:15	10.65±0.49

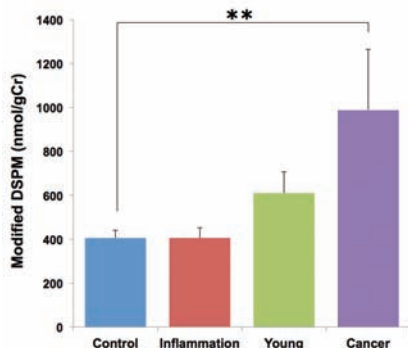


Figure 1. Urine diacetylated spermine concentration in each group (control vs young, **P<0.05).

Statistical analysis

Statistical analysis was performed using commercial statistical software (Stat view 4.0). Statistical analyses were performed with ANOVA with *Bonferroni* test. A P value <0.05 was considered a significant difference.

Results

There was a significant elevation in the urine diacetylated spermine concentration in the cancer-bearing group, compared to that in the control group (P=0.0081). Further, the urine diacetylated spermine concentration in the cancer-bearing group did not differ markedly from that in the young group (P=0.24). However, there was statistic significant different between control and young group dogs (P=0.019) and between the inflammatory and cancer-bearing groups (P=0.62).

Discussion

In this study, the urine diacetylated spermine concentration of the tumor-bearing group only showed a significant elevation compared to the control group. In general, polyamine concentrations are dependent on age,⁹ because they are present in all prokaryotic and eukaryotic cells associated with growth processes.¹⁰ However, there was no significant elevation in young dogs the compared to control group, although the concentration tended to increase as shown in Figure 1. This phenomenon might be associated with systemic cellular proliferation. Taken together, although we did not evaluate the age-dependent elevation of urine diacetylated spermine, we suggest that diacetylated spermine is not useful as a tumor marker in dogs under the age of 2 years. It was reported that the urine diacetylated spermine concentration was elevated in acute appendicitis, cholecystitis, inflammatory bowel disease, viral load, steroids, and low calorie diet.^{11,12} However, there was no significant elevation in inflammation compared to the control group in our study. To the best of our knowledge, there was no previous report on the association among CRP, WBC count, and diacetylated spermine. Thus, the dissociation recognized in our study could not be com-

pletely explained.

On the other hand, urine diacetylated spermine was significantly elevated in patients with cancer.³ In the present study, we found a significant elevation in the cancer-bearing group. We selected malignant tumors for the cancer-bearing group, because in the comparison study between malignant and benign bladder tumors in humans.⁸ Thus, it was suggested that the concentration of urine diacetylated spermine might depend on tumor type and malignancy/benignity. Although we did not evaluate these parameters in the present study, veterinarians should consider the sensitivity and specificity of the examination method. These are statistical measures of the performance of a binary classification test. Sensitivity measures the proportion of actual positives that are correctly identified as such. Specificity measures the proportion of negatives that are correctly identified. In the near future, we shall perform further study to elucidate these parameters of urine diacetylated spermine concentration. However, a polyamine biosynthesis inhibitor has been used for human cancer patients in a phase I study;¹³ thus, the diverse research on polyamines will lead to the development of new anti-cancer drugs and non-specific tumor markers in the future.

Conclusions

In our study, the urine diacetylated spermine concentration was significantly elevated in cancer-bearing dogs, and this may be clinically useful in confirming the presence of canine tumors to the owners. However, further study in the near future is warranted.

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