NOTE *Public Health*

The Silicon Concentration in Cat Urine and Its Relationship with Other Elements

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ABSTRACT. To understand the effects of silicon (Si) in the urine with respect to the formation of urinary stones, the distribution of Si in urine was observed. Urine samples from cats with urolithiasis (n=10) and healthy cats (n=15) were used. The concentration of Si in the cats with urolithiasis was significantly higher ($P<0.001$). A significant correlation ($P<0.05$) was observed between the concentration of Si and those of other elements, such as calcium, magnesium, phosphorus, potassium and iron, only in the urine of the healthy cats. The distribution of elements in the urine differed between the cats with urolithiasis and the healthy cats. The Si concentration and its relationship with other elements were suggested to be useful biomarkers for urolithiasis in cats.

KEY WORDS: biomarker, feline, silicon, urine, urolithiasis.

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There is an important area related to human health in the field of veterinary science: the development of animal models for various diseases is an appropriate study. Furthermore, case analyses obtained from veterinary clinic sometimes provide a variety of solutions for diseases that affect humans. In the study of urolithiasis, there are many reports describing the various types of urolithiasis, such as uric acid urolithiasis, struvite calculi and calcium oxalate stones. Various epidemiological aspects, such as sex differences [11], overweight [13] and breed [16] or races [2], have been indicated as factors in stone formation. Furthermore, there are interesting reports of the relationships between elements in foods and the pH of the urine [15] and of the relationship between the mineral balance of the food and renal function [1]. However, there are few reports that describe the relationship between the concentrations of elements and clinical cases of urolithiasis. Thus, eight elements, calcium (Ca), cupper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) and sulfur (S) were observed, and their relationships with urolithiasis were investigated in a previous study using urine of cats [7]. A significant correlation was obtained among various elements in the urine of healthy cats, although a similar correlation was not obtained in the urine of cats with urolithiasis. Then, the authors suggested that the results obtained from cats may have the potential to develop a model for human disease [7].

On the other hand, Si excess is known to lead to the formation of urinary calculi [14]. In particular, urolithiasis caused by silicon dioxide has been reported in humans [5], pet animals [5] and domestic animals [4]. Furthermore, silicon (Si) is found in other types of urinary stones, such as struvite and calcium oxalate stones [4]. As mentioned above, it was thought that Si is an element that may influence stone formation in urolithiasis. However, the authors could not find a sufficient number of adequate reports describing the Si concentration in the urine of both humans and animals. Thus, in the present study, we investigated the Si concentration and its relationship with other elements in urine.

A total of 39 samples of urine were collected from several veterinary hospitals in Japan. The urine samples were collected using cystocentesis, abdominal compression and urethral catheters. The frozen samples were transferred to Nippon Veterinary and Animal Science University on ice condition. A total of 25 samples were usable in this study, as

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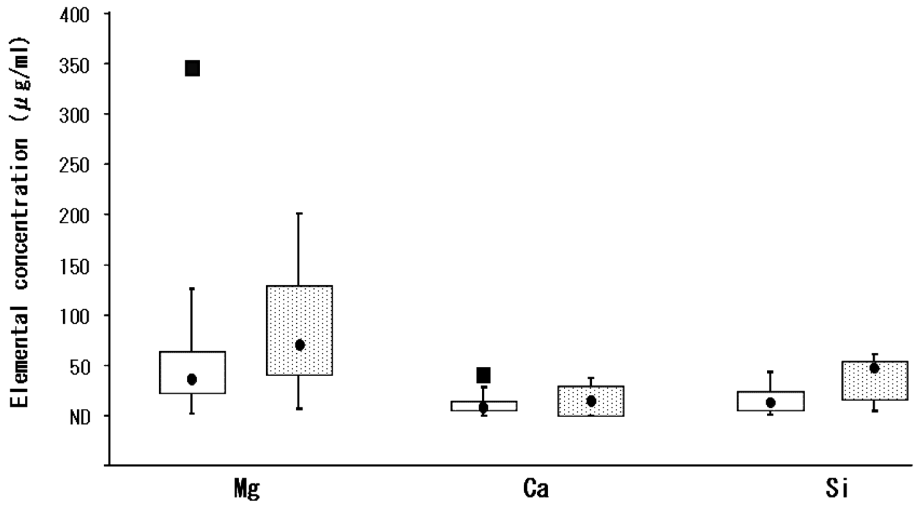

Fig. 1. The concentrations (μg/m*l*) of elements in the urine of healthy cats (n=15, white square in the figure) and cats with urolithiasis (n=10, dotted square in the figure). The concentrations of Ca, Mg and Si were not shown in our previous reports, although the data for K, Na, P and S were presented in our previous report [7]. Black square; outlier, black circle; median.

the medical records were flawed in several of the samples. As a result, 15 of the urine samples were thought to be from healthy cats, according to the data in the medical records, and 10 of the urine samples were thought to be from cats with urolithiasis, such as struvite (n=5), apatite (n=3) and mix of struvite and apatite (n=2). An aliquot of 2 m*l* of each whole urine sample was transferred into a Pyrex tube (Iwaki Glass Co., Chiba, Japan), combined with a 1 ml of mixture of acids (1:1 nitric acid (HNO_3) and perchloric acid, Wako Pure Chemical Industries, Osaka, Japan). The all samples were put into thermo block and digested at 180°C. The samples were evaporated to dryness. Just before analysis, each sample was centuplicated by 0.1N HNO_3. The determination of elements was performed using inductively coupled plasma emission spectrometry (FTP-08, Spectro A.I., Kleve, Germany). The target elements in this study were calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), silicon (Si) and sulfur (S). The information of age, sex and breed in medical records was used as epidemiological studies. Details about the information and preparation methods of the samples were provided also in our previous reports [7].

The data were analyzed using Lotus 2001 (Lotus Development, Cambridge, MA, U.S.A.), Excel 2010 (Microsoft Japan, Tokyo, Japan) and JMP (SAS Institute, Tokyo, Japan). The concentration of Si in the urine was represented by the mean values and the standard error of the mean (SEM). Significant differences between the contents were analyzed using Student's *t*-test. The significance of Pearson's correlation was tested using software (SPSS 19, IBM Japan, Ltd., Tokyo, Japan), and the differences in the regression lines were tested using add-in software for Excel (Esumi Co., Ltd., Tokyo, Japan).

In the present study, the concentration of Si in the urine of cats with urolithiasis was compared with that of healthy cats. The method of sample processing in the present study was an issue that required exploration, as glass and quartz tubes contain silica as a major ingredient [8, 12]. When the blank and urine samples were prepared on a like-for-like basis, the mean Si concentration of the blank sample was 0.014 μg/m*l* (n=5). Because the amount of Si eluted from the glass was small and urine samples have a comparatively high Si concentration, it was concluded that this issue presented no major problem for the analysis of Si in urine.

The mean Si concentration of normal urine was 15.31 ± 3.13 μg/m*l* (Fig. 1). This value is higher than the results of cats (concentration of silica dioxide, 3–8 mg/100 m*l*) from other reports [3]. The higher result in our study was thought to be due to sample preparation of urine. Sample preparation, such as centrifugal treatment, was not used in our previous study [7] and this study, because it was thought the differences become glaring using urinary precipitate. In a further study, the comparison of centrifugal filtrated urine is also necessary.

Because various epidemiological aspects are thought to influence the concentration of elements, the elemental concentration, such as Ca, Cu, Fe, K, Mg, Na and P, in normal urine was investigated by classification of several epidemiological aspects in our previous reports [7]. Similar investigation was performed using normal urine. The differences in sex (male (n=7 including 4 castrated males); 14.51 ± 5.27 μg/m*l*, female (n=8 including 3 neutered females); 15.84 ± 4.11μg/m*l*), food type (dry food (n=4); 13.24 ± 4.41μg/m*l*, dry and wet food (n=6); 14.11 ± 6.86μg/m*l*, wet food (n=2); 16.01 ± 3.34 μg/m*l*, unknown (n=3); 20.00 ± 7.00 μg/m*l*), feeding period, condition of breed (inside (n=7);10.77 ± 3.72 μg/m, outside and inside (n=3);24.47 ± 9.76 μg/m, unknown (n=5);16.17 ± 5.01 μg/m), age (more than 1 year (n=8); 16.08 ± 5.41 μg/m, less than 1 year (n=6);15.05 ± 3.61 μg/m, unknown;10.72 μg/m) and breed (half-breed;14.63 ± 3.44 μg/m*l*, purebred;16.67 ± 6.99 μg/m*l*) did not influence the

Si concentration of normal urine in the present study. This tendency was similar to that observed in our previous report investigating Ca, Cu, Fe, K, Mg, Na and P [7].

In the present study, the concentration of Si in the urine of cats with urolithiasis was compared with that of healthy cats (Fig. 1). Although the cats with urolithiasis used in this study were not affected with silica urolithiasis, the concentration of Si in the urine of the cats with urolithiasis (mean Si concentration, 39.63 ± 6.38μg/m*l*) was significantly higher (*P*<0.001) than that in the urine of the healthy cats. On the other hand, the ratio between Si and other elements was different between urine of healthy cats and that of cats with urolithiasis. The ratios between Si and other elements, such as Ca (ratio; 0.39), Mg (2.18), P (39.15), K (60.17) and S (56.27), in urine of cats with urolithiasis were lower than those of urine of healthy cats (ratio, Si-Ca; 0.79, Si-Mg; 3.93, Si-P; 66.51, Si-K; 89.90 and Si-S; 84.17). This finding suggested that Si concentration is relatively high in the urine of cats with urolithiasis. Thus, since it was thought that the distributions of elements are different between normal urine and abnormal urine, the interaction of elements was investigated in the next experiments. There was a significant correlation (r=0.8020, *P*<0.01) between the concentration of Si and that of Ca in the urine of cats with urolithiasis, although the obtained correlation between the concentrations of Si and Ca was not significant (r=0.0599, *P*=0.8321) in the urine of healthy cats (Fig. 2). It has been reported that the Si appeared and increased in the active growth areas of bone before maturity [9]. As mineralization progresses, Si might be increased concomitantly with Ca. Thus, it is thought to be a close connection between Si and Ca concentration in metabolism of animals. In contrast, a significant correlation (*P*<0.05) was found between the concentration of Si and that of Mg in the urine of the healthy cats, while the same correlation was not observed in the urine from the cats with urolithiasis. The evidence for the important role of urinary Mg to restrict solubility of Si in urine has been reported by Beeson *et al.* [9]. Thus, while the low incidence of Si urinary stone, our findings suggest that the higher Si in the urine may be a substance involved in the formation of urinary stones. In fact, it has been reported that Si can also be detected in urinary stones, such as struvite and calcium oxalate stones [17].

On the other hand, there were significant correlations between the concentrations of Si and S in the urine of both healthy cats (r=0.6434, *P*<0.05) and cats with urolithiasis (r=0.7001, *P*<0.05) (Fig. 3). Because the data varied widely, there was no significant difference between the regression line of the healthy cats and that of the cats with urolithiasis. This phenomenon should be discussed continuously.

A significant correlation (*P*<0.05) was found between the concentration of Si and that of other elements, such as P, S and K in the urine of the healthy cats. In a previous study, we established a new index of Cd contamination in animals that indicates normal metabolism of animals including humans [6]. This index, a regression line, was generated using the mean Cd contents of the kidney and liver of animals that were thought not to be contaminated with Cd. The data obtained from abnormal animals, such as experimental animals treated with Cd and Cd-poisoned humans, were separated from this index. Thus, the loss of equilibrium in abnormal animals was suggested in this previous report. Since the similar correlation was not observed in the urine from the cats with urolithiasis, it was thought that a similar finding would be obtained in the investigation of multiple elements using urine. Interestingly, after the conversion of the concentrations of elements to normal logarithms in the urine from the healthy cats, the several combinations of elements were classified into two types of regression line (Fig. 4). As shown in figure, two regression lines were obtained due to different ratio of elemental concentration. Thus, Y intercept of the regression line was significantly different (*P*<0.01).

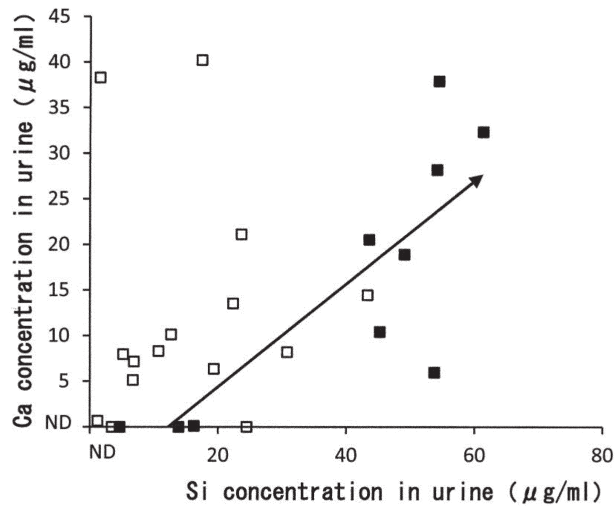

Fig. 2. The correlation between the concentrations of Si and Ca in the urine of cats: healthy cats (empty squares and dotted line, n=15) and cats with urolithiasis (filled squares, n=10).

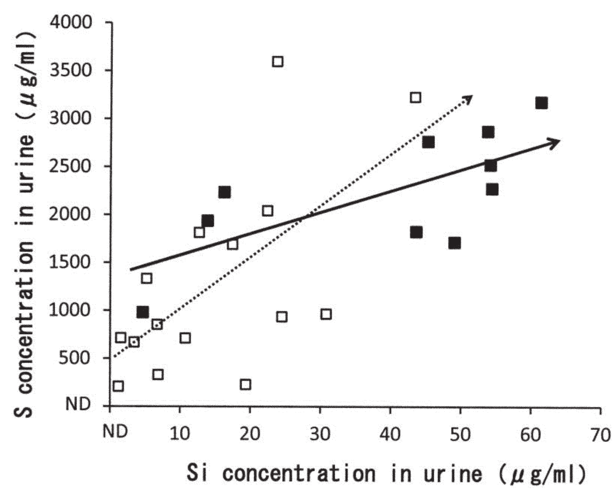

Fig. 3. The correlation between the concentrations of Si and S in the urine of cats: healthy cats (empty squares and dotted line, n=15) and cats with urolithiasis (filled squares and solid line, n=10).

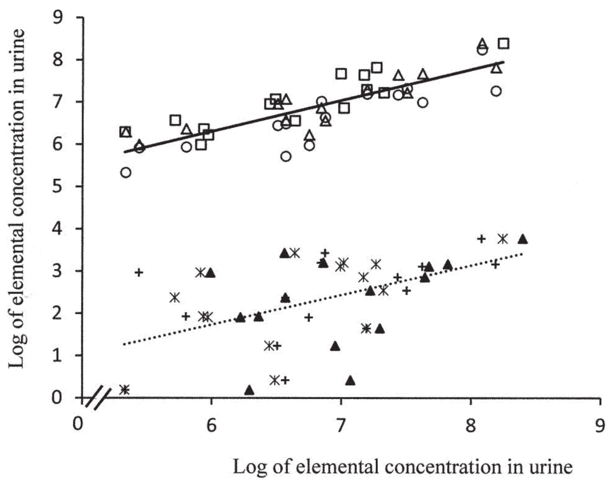

Fig. 4. The relationships between multiple elements after logarithmic transformation. The combinations of S-K (empty triangles), S-P (circles), P-K (squares), P-Si (asterisks), K-Si (filled triangles) and S-Si (crosses) are shown. The first variable of each pair is measured on the abscissa axis and the second on the ordinate axis.

However, since the slope of regression line was not different (P=0.862), it was thought that the slope of regression line indicates equilibrium of interaction of elements. The finding in urine of cats suggested that an equilibrium is also established in normal urine.

In a survey of silica urolithiasis in dogs, it was suggested that male dogs are more likely to be affected than female dogs [10]. The type of food was also suggested to be a possible factor in silica urolithiasis. It was suggested that the prevalence of silica urolithiasis in dogs has increased since the mid-1970's and the grain added to commercially available pet food was generally cited as a factor in silica urolithiasis [17]. The authors could not find the studies describing the concentration of Si in urine of cats. Thus, our study is thought to be one of knowledge to understand the urolithiasis of cats. Further, the different trends between cats with urolithiasis and healthy cats suggested that the investigation of the Si concentration in urine is a possible biomarker for understanding urolithiasis. Since the findings in the present study will be also useful knowledge for human health, this study was thought to be a valuable study of public health in veterinary science.

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