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Original Article

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Abstract

Blood pressure, urinary protein creatinine (UPC) ratio and oxidative stress (plasma melondialdehyde, urinary melondialdehyde creatinine ratio; UMDA/Cr, the activities of red blood cell catalase; RBC-CAT and red blood cell glutathione) were studied in 42 dogs with urolithiasis and 14 control healthy dogs. The types and location of calculi were defined. The results showed that calculi preferentially found in male and female dogs were calcium oxalate and struvite, respectively. In female, calculi found mostly at urinary bladder while in male, they could be found in either urinary bladder and/or urethra. Dogs with urolithiasis were normotensive. However, UPC ratio was higher significantly (p<0.001) compared with control dogs. The BUN and plasma creatinine concentrations were elevated in dogs with urolithiasis only if the obstruction occurred especially in dogs with renal calculi. Dogs with urate urolith had lower BUN compared with control dogs (p<0.05). There were no significant changes in oxidative stress parameters among each type of urolith except lower red blood cell catalase activity in calcium oxalate urolith (p<0.01). The significant positive relationships between UMDA/Cr and either UPC ratio (p<0.01) or plasma creatinine concentration (p<0.05) were found. The results suggest that the oxidative stress may occur only in dog with calcium oxalate urolith. The renal oxidative damage which was represented by UMDA/Cr could be elevated when renal impairment was developed.

Keywords: Blood pressure, dogs, oxidative stress, UPC ratio, urolithiasis

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บทคัดย่อ

ความดันเลือด สัดส่วนการขับทิ้งโปรตีนต่อครีอะตินีนในปัสสาวะและความเครียดออกซิเดชั่นในสุนัขที่เป็นนิ่วทางเดินปัสสาวะ

สุภา สิทธานุกูล[1] ปรมินทร์ ฉายารัตนศิลป์[1] วิมลภา หิรัญประดิษฐ์[1] วินัย ชาญสายสาคร[2] มนคน ตรีศิริโรจน์[2] ศิริเพ็ญ โกมลวานิช[1] ทรายแก้ว สัตยธรรม[1] ชลลดา บูรณกาล[1]*

วัตถุประสงค์เพื่อศึกษาความดันเลือด สัดส่วนการขับทิ้งโปรตีนต่อครีอะตินีนในปัสสาวะและความเครียดออกซิเดชั่น (ปริมาณของเมลอนอัลดีไฮน์ในเลือดและปัสสาวะและการทำงานของเอ็นไซม์คาทาเลสและกลูตาไทโอนในเม็ดเลือดแดง) ในสุนัขที่เป็นนิ่ว 42 ตัว เปรียบเทียบกับสุนัขในกลุ่มควบคุม 14 ตัว โดยศึกษาตำแหน่งและชนิดของนิ่ว จากผลการศึกษาว่า นิ่วที่พบมากที่สุดในเพศผู้และเพศเมียเป็นชนิดแคลเซียมออกซาเลตและสตรูไวท์ตามลำดับ ในเพศเมียมักพบนิ่วในกระเพาะปัสสาวะแต่ในเพศผู้พบทั้งในกระเพาะปัสสาวะและท่อทางเดินปัสสาวะ สุนัขในกลุ่มที่เป็นนิ่วในทางเดินปัสสาวะมีค่าความดันเลือดปกติ แต่ สัดส่วนการขับทิ้งโปรตีนต่อครีอะตินีนในปัสสาวะสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ($p<0.001$) ค่าครีอะตินีนและยูเรียไนโตรเจนในเลือดอยู่ในเกณฑ์ปกติยกเว้นมีการอุดตันทางเดินปัสสาวะโดยเฉพาะสุนัขที่เป็นนิ่วที่ไต ค่ายูเรียไนโตรเจนในเลือดจะต่ำกว่าปกติในสุนัขที่มีนิ่วยูเรต ($p<0.05$) อย่างไรก็ตามไม่พบการเปลี่ยนแปลงความเครียดออกซิเดชั่นนอกจากการลดลงของคาทาเลสในเม็ดเลือดแดงในสุนัขที่เป็นนิ่วชนิดแคลเซียมออกซาเลต ($p<0.01$) พบความสัมพันธ์เชิงบวกระหว่างการขับทิ้งเมลอนอัลดีไฮน์และโปรตีนในปัสสาวะ ($p<0.01$) และระหว่างการขับทิ้งเมลอนอัลดีไฮน์ในปัสสาวะและครีอะตินีนในพลาสม่า ($p<0.05$) ผลการทดลองบ่งชี้ว่าเกิดความเครียดออกซิเดชั่นในสุนัขที่เป็นนิ่วเฉพาะชนิดแคลเซียมออกซาเลต ส่วนการเกิดความเครียดออกซิเดชั่นที่ไตโดยประเมินจากการขับทิ้งเมลอนอัลดีไฮน์ในปัสสาวะ จะพบก็ต่อเมื่อเกิดการบกพร่องของการทำหน้าที่ของไตแล้ว

คำสำคัญ: ความดันเลือด สุนัข ความเครียดออกซิเดชั่น สัดส่วนการขับทิ้งโปรตีนต่อครีอะตินีนในปัสสาวะ นิ่วทางเดินปัสสาวะ

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Introduction

Urolithiasis is one of the common problems found in dogs. The syndrome of urolith may involve familial, congenital or acquired pathophysiologic factors which increased risk of precipitation of some metabolites in urine to form stone (Osborne et al., 2008). The common place of urolith found in female is urinary bladder while in male some of the stone may be obstructed in the urethra. Some urolith occurred in kidney and urinary tract higher than the bladder and may be a cause of severe azotemia and renal impairment. The type of urolith may be varied depending on age, breed, sex, anatomic variation, diet, metabolic defect, urine pH, and whether infection occurs. Some stone such as urate stone was commonly found in Dalmatian (Bartges et al., 1999). From the data collecting between 1981 to 2007 in almost 350,000 stone from Minnesota Urolith center, the prevalence of calcium oxalate was approximately equal to struvite in the past few years (Osborne et al., 2008). The most common signs of dogs with urinary calculi were stranguria, dysuria and may lead to azotemic sign such as vomiting if the urinary tract has been obstructed.

The renal function as shown by BUN and plasma creatinine may be normal in dogs with urolith if no urinary obstruction occurred. Fractional excretion of electrolytes may become normal if tubular function was preserved. Thus, renal function may not be decreased as long as the post-renal obstruction and UTI were not affected glomerular filtration. Rather than renal functional changes in chronic kidney disease, animals may develop the high blood pressure and proteinuria. As part of pathophysiology of stone formation, the blood pressure and UPC ratio have not been studied extensively. Previous work showed that hypertensive human had higher risk to develop kidney stone twice that of normotensives (Cappuccio et al., 1990). In animal model, the spontaneous hypertensive rats, the incidence of kidney stones parallels the severity and

chronicity of hypertension (Wexler and McMurtry (1981). However, no such data available relating the blood pressure and stone formation in dogs with varying types and locations of stone. Moreover, the urinary protein excretion as expressed in UPC ratio may increase by mean of post-glomerular proteinuria as infection develops in urinary tract (Lulich and Osborne, 1994).

Free radical can be produced during infection or in many disease circumstances. If the oxidants was generated more than the body can eliminated by antioxidant defense mechanism, oxidative stress with the elevation of free radicals may occur and subsequently injure cellular tissue especially proteins, DNA and lipids resulting in cell degeneration. The imbalanced between the pro-oxidants and antioxidants can be quantified by measuring the redox state of reduced glutatione (GSH), the activities of antioxidative enzymes such as catalase, glutathione peroxidase and superoxide dismutase and the products of lipid peroxidation, melandialdehyde (MDA). The capacity of antioxidant will determine whether the oxidizing agents were completely destroyed and eliminated from the body in order to restore normal cell function. In human, the prevalence of renal stone contributed the renal disease is worldwide. The stone is composed mainly of calcium oxalate. The oxalate or calcium oxalate monohydrate crystal induced production of free radical has been observed in LLC-PK1 and MDCK cells which can be alleviated by many antioxidants including vitamin E (Thamiselvan et al., 2000; Thamilselvan et al., 2003). Experimental induced hyperoxaluria by ethylene glycol ingestion showed increased calcium oxalate excretion, crystal deposition and renal tubular damage along with increased lipid peroxidation (Thamiselvan et al., 1997). Moreover, patient human with calcium oxalate urolith had high lipid peroxidation with increased urinary N-acetyl-β-glucosaminidase (NAG) activity (Tungsanga et al., 2005). In dogs, the urolith appear in many locations and the compositions were variable. No available data regarding oxidative damage in any kind of urolith were shown and whether any relationships occur between oxidative stress and plasma BUN, Cr, blood pressure or UPC ratio in dogs with urolithiasis.

Materials and Methods

The study was performed in accordance with institutional guideline and conformed to the Faculty of Veterinary Science, Chulalongkorn University. Fourteen healthy dogs that serve as control group and forty two dogs with urolthiasis (urolith group) regardless of age, sex, breed and the episode of stone formation were recruited in this study. The survey radiography was used to diagnose dogs with urolithiasis. Additional contrast radiography and ultrasonography were performed in some cases. The urolithiasis dogs were dogs that have stone regardless of location of stone formation. The upper urinary tract stone was defined as the stone in which the location was in the renal tubule inside the kidney, renal pelvis or ureter while the lower the urinary tract stone was the stone in the urinary bladder and the urethra. Some dogs had the stone at both locations in the same episode. The analysis of stones which were removed after surgery were performed at the Urolith Center, College of Veterinary Medicine, University of Minnesota.

All dogs in this study were not received any prior antioxidants such as vitamin A, C and E. An indirect blood pressure was performed in all dogs at the upper hock joint using oscillometric technique (Fukuda, Tokyo, Japan). One millilitre of blood was collected in sodium heparinized tube for the determinations of blood urea nitrogen (BUN), plasma creatinine concentrations and packed cell volume. Additional three milliliters of blood were collected, plasma was separated for measurement of plasma melondialdehyde (P-MDA), Na and K concentrations. The remaining erythrocytes were washed and kept for determination of the concentrations of red blood cell catalase (RBC-CAT) and glutathione (RBC-GSH) as described previously (Buranakarl et al., 2009).

Urine was collected for urinalysis and determination of urinary concentrations of MDA (U-MDA), protein, creatinine and electrolytes (Na and K).

Analytical methods: The packed cell volume was determined using microcentrifugation method. Concentrations of blood urea nitrogen and creatinine were measured using automated analyzer (BT2000, Biotecnica Instrument, SPA, Rome, Italy). Plasma and urinary concentrations of Na and K were measured by flame photometer (Flame photometer, 410C, Ciba Corning Diagnostic Scientific Instruments, Halstead, UK). The urinary fractional excretion of both electrolytes were calculated using the ratio of clearance of electrolyte to that of creatinine. Urinary protein creatinine ratio was performed by precipitate protein with 3% sulfosalicylic acid and expressed as urinary protein creatinine (UPC) ratio. The urinalysis was performed according to the standard procedure.

Concentration of MDA in plasma and urine was measured by modified method of Ohkawa et al. (1979) based on the reaction with thiobarbituric acid and expressed as nmol/ml and nmol/mgCr, respectively. The urinary MDA was expressed as urinary MDA creatinine ratio (UMDA/Cr). The RBC-GSH was measured according to modification of the method of Beutler et al. (1963) and expressed as nmol/gHb. The RBC-CAT activity was determined by method of Aebi (1983) and expressed as kunit/gHb. One unit of catalase was decomposed of 1.0 μmol of hydrogen peroxide to oxygen and water per minute at a substrate concentration of 10 mM hydrogen peroxide. The hemoglobin concentration was obtained by method of Drabkin (Frankel et al., 1970).

Statistical analysis: Results are expressed as mean±SEM. Different in mean between control and urolith groups were compared using student unpaired t-test or Mann-Whitney rank sum test. Linear regression and Pearson correlation were used to determine the relationship between variables. Significance was considered when *p*-values were less than 0.05. All statistical analysis were performed using SigmaStat program.

Results

The study was performed in 23 males and 19 females dogs with urolithiasis with the age between 4-14 year old while the control healthy group consisted

of 14 dogs with the age between 1-9 year old. Most of the dogs with urolithasis had clinical signs of poikiuria, dysuria, difficult and prolonged urination while some of them had hematuria. The clinical findings were not different among stone type. However, signs are related to the location and the potential of stone obstruction. The 88% of the urolithiasis cases had the stone in the lower urinary tract. Only two dogs had stone in only the upper urinary tract. One dog had the stone in the ureter which composed of struvite and another had the stone in the kidney but surgery was not performed. Three cases had the stone in both the upper and lower urinary tract. One dog showed the calcium oxalate urolith in urinary bladder and the uric acid stone in the kidney. This dog was previously operated by cystostomy to remove the calcium oxalate stone 10 months earlier. One dog in this group did not have operation to remove the stone while another dog had stone removal which revealed struvite stone in the bladder and calcium oxalate stone in the kidney. Among 37 dogs that had the stone only in the lower urinary tract, the stones were removed and the composition of the stone was analyzed in 29 of them. The incidence of struvite, calcium oxalate, urate and mixed type stones were 62.1%, 20.7%, 10.3% and 6.9%, respectively.

Among 42 dogs in this study, the calculi was found for first episode by 73.8% while the second and third episodes were found in 21.4% and 4.76% of cases, respectively. The types of calculi in male and female dogs were shown in table 1. It was shown that male dog usually had calcium oxalate urolith while female dogs had struvite calculi.

Table 1 Percentage of urolith type found in male and female dogs

	Struvite	*CaOX*	*urate*	*mixed*
Male (n=16)	31.3	43.8	18.7	6.2
Female (n=15)	93.3	-	-	6.7

The location of the stone in male and female dogs was shown in table 2. Most of the calculi were found in urinary bladder in female dog while they can be found in both urethra and bladder in male dogs.

Table 2 The location of stone found in male and female dogs

Location	*Male (%)*	*Female (%)*
Kidney (n=1)	4.35	-
Ureter (n=1)	-	5.26
Urinary bladder (n=24)	43.38	73.68
Urethra (n=3)	13.04	-
Kidney & urinary bladder (n=3)	8.7	5.26
Urinary bladder & urethra (n=10)	30.43	15.8

The urinalysis results showed that struvite stone can be found in acidic, neutral or basic urine pH. The average urine pH for all stone type was 6.71±0.22 while urine specific gravity was 1.027±0.002. Most of the struvite and calcium oxalate stone had positive results of protein and blood on urine strip test.

Blood pressures in dogs with urolithiasis regardless of stone type were shown in table 3. The systolic, diastolic and mean blood pressure showed no differences in dogs with urolithiasis and among all stone type except the systolic pressure in dogs with mixed type urolith that had systolic pressure higher than control (p<0.05).

Table 3 Blood pressure and UPC ratio in dogs with urolithiasis

	Control	*Urolithiasis*
SAP(mmHg)	131.6 ± 6.2 (14)	131.3 ± 4.6 (39)
MAP(mmHg)	100.7 ± 5.3 (14)	99.2 ± 3.8 (39)
DAP(mmHg)	78.6 ± 5.0 (14)	76.8 ± 3.4 (39)
UPC ratio	0.26 ± 0.13 (11)	3.72 ± 1.11*** (40)
FENa(%)	1.61 ± 0.46 (12)	1.39 ± 0.26 (36)
FEK(%)	14.12 ± 3.88 (12)	12.48 ± 2.43(36)

Values are presented as mean ± SEM, number in parenthesis indicated number of dogs.

SAP: systolic arterial pressure, MAP: mean arterial pressure, DAP: diastolic arterial pressure, UPC ratio: urinary protein creatinine ratio, FENa: fractional excretion of sodium, FEK: fractional excreation of potassium

*** p< 0.001 using Mann-Whitney-Rank Sum test

Plasma creatinine and BUN were within normal limits in dogs with lower urinary tract stone. One dog with only renal calculi had azotemia with BUN of 45 mg/dl and creatinine of 1.7 mg/dl. Three dogs with both upper and lower urinary tract calculi had significant increase in plasma creatinine concentration (2.43±0.72 mg%, p<0.01) compared with control healthy group (1.02±0.10 mg/dl). The BUN was also higher although not significant difference (53.0±22.0 vs 19.8±1.4 mg/dl). The PCV in this group was significantly lower than in control group (38.7±4.1 vs 47.7±1.4, p<0.05).

Dogs with urate urolithiasis had the significantly lower BUN (8.5±2.5 mg/dl, n=3) compared with control group (p <0.05). The PCV was low in dogs with CaOx urolith (42±2.6 %, n= 7, p<0.05) and in urate urolith (35.3±1.2%, p<0.01) compared with control group. The overall PCV in urolith group regardless of stone type was 42.5±1.2% which was significantly lower than control (p<0.05).

High UPC ratio was found in all groups that had stone regardless of location. The UPC ratios in control was 0.25±0.13 (n= 14) which was lower than upper, lower and both location (3.26±3.15, n=2, 3.83±1.26, n= 34, p<0.001 and 2.67±1.88, n= 3, p<0.05, respectively). Dogs with struvite stone had the highest UPC ratio (5.19±2.33, n= 19, p<0.001). The UPC ratio was also high in CaOX stone (2.15±0.85, n= 7, p<0.01). No change of UPC was found in dogs with urate or mixed stone. Overall UPC ratio of dogs with urolithiasis was higher significantly than control (p<0.001) (table 3). Fractional urinary excretions of Na and K were not different regardless of location or type of stone. The excretions in all dogs with urolithiasis were shown in table 3.

The oxidative stress parameters were shown in table 4. P-MDA, UMDA/Cr and RBC-GSH were not differences in urolithiasis group compared with control group regardless of type of calculi. Dogs with stone composed of calcium oxalate urolith had the RBC-CAT activity lower than control dogs (p<0.001). However, the overall RBC-CAT activity in dogs with urolith were not different than control.

Table 4 Oxidative stress in different type of calculi

Type	*P-MDA (nmol/ml)*	*UMDA/Cr (nmol/mgCr)*	*RBC-CAT (U/gHb)*	*RBC-GSH (µmol/gHb)*
Struvite	8.59 ± 1.26 (19)	13.18 ± 2.20 (17)	5149 ± 794 (17)	52.24 ± 2.41 (18)
CaOX	8.74 ± 1.13 (7)	14.31 ± 4.25 (7)	1902 ± 323 (7)**	51.74 ± 4.24 (7)
Urate	8.53 ± 1.50 (3)	12.06 ± 1.94 (3)	2739 ± 800 (3)	50.63 ± 2.16 (3)
Mixed	10.37 (1)	15.08 ± 8.18 (2)	3638 ± 1053 (2)	45.47 ± 2.26 (2)
Urolithiasis	8.26 ± 0.65 (41)	15.40 ± 1.70 (40)	3996 ± 424 (39)	50.64 ± 1.63 (41)
Control	7.29 ± 0.80 (13)	13.99 ± 2.84 (12)	3992 ± 368 (14)	49.21 ± 2.03 (14)

Values are presented as mean ± SEM, number in parenthesis indicated number of dogs.

P-MDA: plasma melondialdehyde, UMDA/Cr: urinary melondialdehyde and creatinine ratio, RBC-CAT: red blood cell catalase, RBC-GSH: red blood cell glutathione, ** p< 0.01 using unpaired t-test

The relationships between oxidative stress parameter and plasma creatinine concentration, UPC ratio and blood pressure were determined in all dogs. By linear regression analysis, it was found that the UMDA/Cr in all dogs was correlated with both UPC ratio (UMDA/Cr = 0.588 UPC+13.162, r= 370, p<0.01, n= 51) and plasma creatinine concentration (UMDA/Cr = 4.534 PCr+10.161, r= 0.335, p<0.05, n =49) (Figures 1a and 1b). No relationships were found between other oxidative parameters and renal function, UPC or blood pressure.

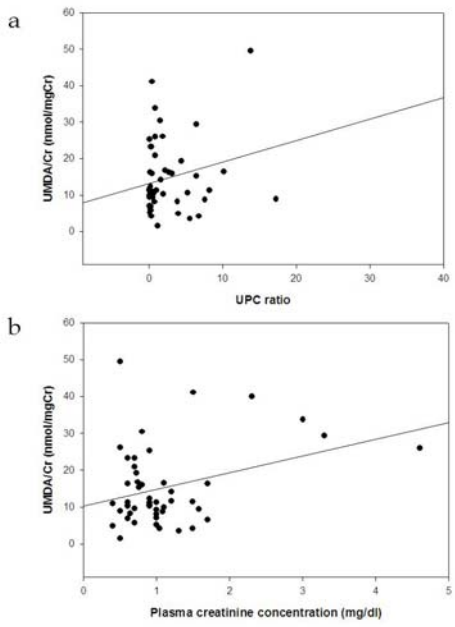

Figure 1 The relationship beween urinary MDA creatinine ratio (UMDA/Cr) and urinary protein creatinine (UPC) ratio (a) and plasma creatinine concentrations (b).

Discussion

The present study was demonstrated that most of the stones were found in the lower urinary tract including bladder and urethra. The composition of calculi was listed on the basis of the predominant mineral that each contained. A predominant mineral was defined as one that was present in an amount >70% of the total mineral contents of the specimen. If different minerals are separated into distinct juxtaposed bands or layers and if one portion of the urolith comprises at least 70% of one mineral type and is surround by one or more layers composed primarily (>70%) of a different mineral, it is classified as a compound urolith (Ulrich et al., 2008). The classification of compound urolith may also be used for urolith composed predominantly (>70%) of different mineral types retrieved from different locations in the urinary tract (Ulrich et al., 2008). In the present study, only 5 dogs had stone in upper urinary tract. The interesting was that the composition of stone found in the upper urinary tract can be either calcium oxalate, magnesium ammonium phosphate (struvite) or uric acid. Moreover, dogs with stone presented in both bladder and kidney could be encountered with different kind of stone in each location. The rationale of treatment may be difficult and complicated in those with multiple types of stone for an instantaneous time.

Dogs with urolith had an age between 4-14 years old with an average of 6-7 years old. Some of these dogs had the stone removal earlier. Struvite uroliths were the most common in female dogs and calcium oxalate uroliths in male dogs. The results were similar to the study by Canadian Veterinary Urolith Center (Houston and Moore, 2009) and from the Gerald V. Ling Urinary Stone Analysis Laboratory at Davis (Low et al., 2010). The prevalence of calcium oxalate urolith was increased over time in which calcium oxalate was detected in only 5% of canine uroliths submitted to Minesota urolith center in 1981 while it was detected up to 41% in 2007 (Osborne et al., 2008). The similar changes in trend of calculi composition were also found from both center in Davis and in Canada (Houston and Moore, 2009; Low et al., 2010). The results suggest the change in diet composition especially animal protein in dog food in the past decade since diet-mediated urine acidification enhances the solubility of MAP crystals in urine while dietary acids promote calcium oxalate crystalluria by inducing hypercalciuria (Osborne et al., 1999).

Urate stone are comprised of the most

frequent stone found in Dalmatian (Ling et al., 1998[a]). Increased plasma uric acid and urinary fractional excretion of uric acid were found (Assarasakorn et al., 2001) although plasma uric acid is normally higher in Dalmatian with or without stone than other breed (Bartges et al, 1999). The liver of Dalmatians does not completely oxidize available uric acid even though it contains a sufficient concentration of uricase (Cohn et al, 1965). Nevertheless, not all of Dalmatian develop this type of stone. In our study two dalmatian had calculi in lower urinary tract and one was analyzed to be struvite. On contrary, an ammonium acid urate can be found in other breeds rather than Dalmatian which may be due to the impaired protein metabolism in liver, portal vascular abnormalities, high purine intake or unidentified cause (Senior, 1992). Our study also showed dog with urate stone had low blood urea nitrogen which may suggest liver malfunction.

The location of stone found in female dogs was urinary bladder while in male dogs, stone can be found in both urinary bladder and urethra. The reason was due to the anatomic difference of the urinary tract in which the male tract is longer than the female and the presence of *os* penis in male. The results were similar to the report of Ling et al. (1998[b]) which showed females were more likely to have urinary bladder calculi than were males while the urethral calculi were more common in male. Franti et al. (1999) studied in specimens from 7,056 female and 6,492 male which showed that the calculi usually located in bladder in both sexes. However, the short tract in female may be venerable for infection than male. In the present study, most of the stones were found in pH between 6 and 7 with the urine specificity of hypersthenuria. The protein and blood were found from the urine strip test in most of these dogs.

Blood pressure in all kinds (systolic, diastolic and mean pressure) were within normal limits in urolithiasis dog compared with control. The blood pressure may not be increased by mean of renal impairment since plasma creatinine concentration and blood urea nitrogen concentration were within normal limit especially dogs that had stone in lower urinary tract. Although male dog had the possibility of stone occlusion in the urethra, the occlusion is partial in this study. Dogs with renal calculi had higher BUN and creatinine since animals were present at the clinic with urinary obstruction with signs of renal insufficiency. The urinary protein creatinine ratio was higher significantly in urolith dogs. Proteinuria in this case may not be due to protein leakage from glomerular basement membrane but rather be due to inflammation of urinary tract. Increased the urinary protein creatinine ratio was found in dogs after experimental induced *E. coli* cystitis for 3 to 4 days (Bagley et al., 1991). It was concluded that the inflammation of lower urogenital system may increase urinary protein creatinine ratio rather than renal origin since the histopathology proved to be normal. Another study in dogs with pyuria and bacteriuria showed increased in albumin but not total protein concentration (Vaden et al., 2004). However, no correlation was found between degree of pyuria and urinary albumin or protein excretions. Thus, proteinuria in pyuria patients may attributable to glomerular disease. In our study, however, urinary tract infection rather than impaired renal function was a cause of high UPC ratio.

Fractional excretion of electrolytes was found to be a good index for tubular function. Increased fractional excretion of Na and K were found in dogs with chronic kidney disease (Buranakarl et al., 2007). However, in the present study the fractional excretion of electrolytes (Na and K) were unaltered. The results were similar to the study in urolithiasis dogs in which fractional excretion of Na and K were normal and similar among each stone type (Assarasakorn et al., 2001).

Although previous study demonstrated that RBC-CAT seems to be lowest in dogs compared with other mammalian species (Szienberg et al., 1969), the red blood cell catalase was the only parameter that altered in urolith dogs. Dogs with stone composition of calcium oxalate had lower RBC-CAT compared with control dog. Tungsanga et al. (2005) studied the oxidative stress in human with kidney stone which comprised of calcium oxalate. They found increased plasma erythrocyte and urinary MDA. The urinary protein and NAG activity were also increased. The erythrocyte glutathione, glutathione peroxidase activity, plasma protein thiol and plasma vitamin E were decreased. The results suggest that oxalate induced tubular cell injury and is mediated by free radical over production. In the present study, the antioxidative enzyme, catalase was decreased only in calcium oxalate urolith group. Although most of the stone was found in lower urinary tract. Calcium oxalate itself may directly stimulate lipid peroxidation. Dogs with calcium oxalate stone had higher urinary calcium and oxalate concentrations, calcium excretion and CaOX relative supersaturation (Stevenson et al., 2004). Previous studies showed that oxalate crystal directly induced cell injury via LPO pathway. Increase production of free radicals has been observed in kidney cell culture incubated with oxalate (Scheid et al., 1996; Thamiselvan et al, 2000) and also in *in vivo* study (Thamiselvan and Khan, 1998). Giving antioxidants, vitamin E, superoxide dismutase, catalase and desferoxamine can provide protection against oxalate toxicity in LLC-PK1 cells (Thamilselvan et al., 2003). However, a study with rats exposed to single intraperitoneal injection of sodium oxalate at the dose of 70 mg/kg showed increased catalase activity in red blood cell along with increased superoxide dismutase activity (Bijarnia et al., 2009). The different results may be due to the time of exposure to oxalate which was only one day in rat study. Although the catalase activity in red blood cell in dogs with calcium oxalate urolith was decreased in the present study, the urinary oxidative marker, urinary MDA and creatinine ratio was unchanged. Dogs with other types of urolithiasis also showed no changes in all oxidative parameters. However, the positive relationships were found between UPC or plasma creatinine with UMDA/Cr. These relationships were also found in the dogs with chronic kidney disease (Buranakarl et al., 2009). It is suggested

that oxidative stress that occurs in urinary system exists when renal impairment or tubular cell inflammation and damage occurs. In conclusion, dogs with urolithiasis had normal blood pressure with increased UPC ratio. The oxidative stress enzyme, RBC-CAT decreased only in dogs with calcium oxalate urolithiasis. However, it is postulated that the urinary oxidative marker UMDA/Cr may increase along with increased plasma creatinine and proteinuria following renal impairment.

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