

## SDS-PAGE Electrophoresis for Urinary Protein Analysis in Dogs with Chronic Kidney Disease and Urinary Tract Infection

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### *Abstract*

The objective of the present study was to investigate urinary protein profiles in dogs with chronic kidney disease (CKD) in comparison to dogs with urinary tract infection (UTI). Animals were divided into 4 groups: control, CKD stages II+III, CKD stage IV and UTI. Blood pressure was measured using oscillometric method. Blood was collected for determinations of packed cell volume (PCV), blood urea nitrogen (BUN) and plasma creatinine concentrations. Urine was collected for urinalysis and protein determination. Total urinary proteins were measured using semi-quantitative method by precipitation with sulfosalicylic acid and a standard SDS-polyacrylamide gel electrophoresis (SDS-PAGE) which were presented as urinary protein creatinine (UPC) ratio and electrophoresis urinary total protein creatinine (E-UTPC) ratio, respectively. The protein of high molecular weight (HMW) (> 67 kDa), middle molecular weight (MMW) (66-67 kDa) and low molecular weight (LMW) (< 66 kDa) were determined. The results showed that blood pressure in dogs with CKD stage IV was significantly higher than the control healthy group ( $p < 0.05$ ). The PCV was lower in dogs with CKD stage IV compared with the control and UTI group ( $p < 0.05$ ). Dogs with CKD stage II+III or IV had significantly higher UPC ratio and E-UTPC ratio ( $p < 0.05$ ) compared with the control group. Although dogs with UTI had higher E-UTPC ratio compared with the control group, it was lower than dogs with CKD stage IV ( $p < 0.05$ ). There were positive correlations between plasma creatinine concentration and both proteinuria (HHW;  $p < 0.01$  and MMW;  $p < 0.05$ ) and mean arterial blood pressure ( $p < 0.05$ ). The urinary protein distributions in CKD groups were similar to UTI. It is concluded that although the proteinuria in CKD was higher than UTI, the degree and pattern of urinary protein with different molecular weight could not be used to distinguish between CKD and UTI in dogs.

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**Keywords:** chronic kidney disease, dogs, proteinuria, SDS-PAGE electrophoresis, urinary tract infection

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## บทคัดย่อ

### การตรวจวิเคราะห์โปรตีนในปัสสาวะโดยวิธีเอสดีเอสเพอเจอเล็กโตรโฟเรซิสในสุนัขที่มีภาวะโรคไตเรื้อรังและภาวะติดเชื้อที่ระบบทางเดินปัสสาวะ

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การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาโปรตีนในปัสสาวะในสุนัขที่เป็นโรคไตเรื้อรัง (chronic kidney disease; CKD) เปรียบเทียบกับสุนัขที่มีภาวะติดเชื้อที่ระบบทางเดินปัสสาวะ (urinary tract infection; UTI) โดยแบ่งสุนัขที่ศึกษาเป็น 4 กลุ่ม คือ กลุ่มควบคุม กลุ่มที่เป็น CKD ระยะ II+III กลุ่มที่เป็น CKD ระยะ IV และกลุ่ม UTI ทำการวัดความดันเลือดโดยวิธีออสซิลโลเมตริก เก็บเลือดนำไปตรวจเม็ดเลือดแดงอัดแน่น (packed cell volume; PCV) ความเข้มข้นของยูเรียไนโตรเจน และครีเอตินีน ทำการวิเคราะห์ปัสสาวะและตรวจหาโปรตีนในปัสสาวะโดยวิธีตกตะกอนกับกรดซัลโฟซาลิไซลิกและทำอิเล็กโตรโฟเรซิสแบบเอสดีเอสเพอเจอ โพลีอะคริลามายด์เจล (SDS-polyacrylamide gel electrophoresis; SDS-PAGE) โดยแสดงผลสัดส่วนปริมาณโปรตีนต่อครีเอตินีนในปัสสาวะในรูป urinary protein creatinine (UPC) ratio และ electrophoresis total urinary protein creatinine (E-UTPC) ratio ตามลำดับ ทำการแยกโปรตีนที่มีน้ำหนักโมเลกุลสูง (high molecular weight protein; HMW) (> 67 กิโลดาลตัน) น้ำหนักโมเลกุลปานกลาง (middle molecular weight protein; MMW) (66-67 กิโลดาลตัน) และ โมเลกุลต่ำ (low molecular weight protein; LMW) (< 66 กิโลดาลตัน) จากผลการศึกษาพบว่า ความดันเลือดในกลุ่ม CKD ระยะ IV มีค่าสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ( $p < 0.05$ ) ค่า PCV ในกลุ่ม CKD ระยะ IV ต่ำกว่ากลุ่มควบคุม และกลุ่ม UTI ( $p < 0.05$ ) สุนัขที่เป็นโรคไตทั้งกลุ่ม CKD ระยะ II+III และ IV มีการเพิ่มสูงขึ้นของ UPC ratio และ E-UTPC ratio ( $p < 0.05$ ) เมื่อเทียบกับกลุ่มควบคุม ถึงแม้สุนัขในกลุ่ม UTI จะมี E-UTPC ratio สูงกว่ากลุ่มควบคุม แต่ค่าที่ได้ก็น้อยกว่ากลุ่ม CKD ระยะ IV ( $p < 0.05$ ) พบความสัมพันธ์เชิงบวกระหว่างความเข้มข้นของครีเอตินีนในพลาสมาและทั้งสัดส่วนการขับทิ้งโปรตีน (HMW;  $p < 0.01$  และ MMW;  $p < 0.05$ ) ต่อครีเอตินีนในปัสสาวะและกับความดันเลือดเฉลี่ย ( $p < 0.05$ ) การกระจายตัวของโปรตีนที่มีน้ำหนักโมเลกุลต่างกันมีรูปแบบคล้ายกันระหว่างกลุ่ม CKD และ UTI การศึกษานี้สรุปได้ว่าปริมาณและรูปแบบของโปรตีนที่มีน้ำหนักโมเลกุลต่างกันที่ตรวจพบในปัสสาวะไม่สามารถนำมาใช้แยกโรค CKD ออกจาก UTI ได้

**คำสำคัญ:** โรคไตเรื้อรัง สุนัข การมีโปรตีนในปัสสาวะ เอสดีเอสเพอเจอเล็กโตรโฟเรซิส การติดเชื้อระบบทางเดินปัสสาวะ

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## Introduction

Proteinuria is one of the significant parameter to verify the progression of kidney disease in human (Iseki et al., 2003). It is also related to survival rate in dogs and cats with kidney disease (Syme and Elliott, 2003; Jacob et al., 2005; Kuwahara et al., 2006). Increased urinary protein loss may be due to abnormal renal handling of protein by the kidney during renal impairment known as renal proteinuria. Categorization of renal proteinuria was performed by ACVIM Concensus Statement in 2005 (Lee et al., 2005). The pathological proteinuria means proteinuria that is attributable to structural or functional lesions within the kidneys. It is also divided into 3 categories; glomerular proteinuria in which the pathologic

lesions occur at the glomerular capillary wall resulting in a change of permselectivity of the capillary. The tubular proteinuria is called when the lesion impairs the tubular recovery of normal plasma proteins that transverse the normal glomerular capillary walls having normal permselectivity properties. Finally, the interstitial proteinuria is called when the lesions cause exudation of plasma proteins from peritubular capillaries into the urinary space. Increased urinary protein loss as measured by UPC ratio, although not related to plasma creatinine concentrations, was found in all stage of renal disease in dogs (Buranakarl et al., 2007). Besides the renal loss of protein, the urinary protein can also be found unrelated to the kidneys. The postrenal proteinuria is due to the entry of protein into the urine after it enters the renal pelvis. The lower urinary tract can be a

source of protein derived from hemorrhagic or exudative processes affecting the wall of urinary excretory pathway as seen in the urinary tract infection (UTI). Report of higher urinary protein creatinine ratio was found in urine of dogs with experimentally induced *E. coli* cystitis (Bagley et al., 1991) whereas increased urinary albumin concentration, but not protein creatinine ratio, was found in dog with pyuria, hematuria and bacteriuria (Vaden et al., 2004). Therefore, the inflammatory process that occurs along the urinary tract can have a profound effect on protein recovery in the urine, making pathologic renal disease undistinguishable from UTI.

Precipitation of urinary protein with sulphosalicylic method is one of the easy procedures to semi-quantify the protein in the urine (Grauer, 2007). Measurements of urinary protein concentration quantitatively with different molecular weight can be performed using electrophoresis (Calzada-Garcia et al., 1996) and is the gold standard for human measurement along with mass spectroscopy (Candiano et al., 2010). Glomerular or tubular proteinuria in relation to kidney disease was associated with urinary recovery of different molecular weight proteins depending upon the mechanism of renal damage (D'Amico and Bazzi, 2003). However, pattern of urinary protein in UTI may be different and related to inflammatory process. Thus, the objectives of this study were to investigate whether E-UTPC ratio is different among control, CKD and UTI dogs; and whether this parameter and urine electrophoresis profile could be used to differentiate the renal and postrenal proteinuria in dogs.

### Materials and Methods

**Animals:** The study was performed in accordance with the ethics committee, Faculty of Veterinary Science, Chulalongkorn University. Dogs that were presented into the Small Animal Hospital, Chulalongkorn University were divided into 4 groups; control healthy group (plasma creatinine concentration; PCr < 1.4 mg/dl without urinary tract infection; UTI), moderated CKD group ( $1.4 < \text{PCr} \leq 5.0$  mg/dl), severe CKD group ( $\text{PCr} > 5.0$  mg/dl) and urinary tract infection (UTI) group. Dogs were diagnosed for chronic kidney disease based on plasma creatinine, blood urea nitrogen (BUN) concentrations, urinalysis results and radiographic/ultrasonographic findings of the kidneys and urinary tract. UTI dog was characterized by a high number of white blood cells in the urine (> 10 cells per high power field; HPF) with or without the presence of bacteriuria. In addition, the plasma creatinine concentrations in UTI group was not elevated ( $\text{PCr} < 1.4$  mg/dl).

Each dog was physically examined by a veterinarian. Blood pressure was measured using oscillometric technique (Fuguda Denshi, Tokyo, Japan). One and a half milliliters of blood was collected into the EDTA and heparinized tubes for determinations of complete blood count (CBC) and

plasma creatinine versus BUN concentrations, respectively. Complete blood count was determined using automate hematology analyzer (The CELL-DYN 3700, Abbott Laboratory, Illinois, USA). PCV was measured by microcentrifugation method. Blood urea nitrogen (BUN), plasma and urinary creatinine concentrations were determined colorimetrically using chemistry analyzer (The IL ILab 650 Chemistry Analyzer, Diamond Diagnostic, MA, USA).

Urine samples were obtained by either voiding or catheterization from each animal for protein and creatinine determination. Urinary protein concentrations were determined by precipitating urine with 3% sulfosalicylic acid and expressed as urinary protein creatinine (UPC) ratio and by electrophoresis methods.

Urine SDS page electrophoresis was run on 12.5% resolving gel and 5% stacking gel which run on a mini protein II Tetra Cell (Bio-rad, NY, USA). The standard protein markers with molecular weight from 10-170 kDa (Strep-tag, Thermo-Fisher Scientific, Miami, USA) were used along with bovine serum albumin (66 kDa). The amount of protein loaded was 2 µg per sample. Gel was stained with Coomassie brilliant blue R-250 Staining Solution Kit (Bio-Rad, NY, USA) and scanned with gel scanner (Visioneer OneTouch 7100, CA, USA). The bands were analyzed and calculated using ImageJ software. The relative percentages of protein fractions were identified using standard albumin with known molecular weight. The total protein yielded from electrophoresis was expressed as electrophoretic urinary total protein creatinine (E-UTPC) ratio. The low, middle and high molecular weight proteins were clarified as protein with molecular weight < 66, 66-67 and > 67 kDa, respectively.

**Statistical analysis:** Data are shown as mean±SEM. Comparisons among all groups were performed using one way analysis of variance (ANOVA) or ANOVA on rank. The relationships between methods of protein measurement and between plasma creatinine concentrations and other parameters were determined using Pearson correlation and linear regression analysis. *P*-value less than 0.05 were considered statistically significant.

### Results

Table 1 shows the number and general characteristics of the dogs in all groups. The mean ages of animals in the control group were less than CKD and UTI groups.

#### *The packed cell volume, plasma creatinine and blood urea nitrogen concentrations and blood pressure*

The PCVs in the dogs with CKD, both stages II+III and IV, were significantly decreased ( $p < 0.05$ ) compared with either the control or UTI groups. The lowest PCV was found in the dogs with CKD stage IV. The PCVs in the control and UTI groups were not different.

**Table 1** General characteristics in 4 groups of dog

| Parameters           | Control                                                                                             | CKD II+III                                                                                                                         | CKD IV                                                                                                    | UTI                                                                                                                              |
|----------------------|-----------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Number of animals    | 9                                                                                                   | 12                                                                                                                                 | 17                                                                                                        | 13                                                                                                                               |
| Breed                | Chihuahua (1)<br>Golden retrievers (2)<br>Mixed (1)<br>Pomeranian (1)<br>Poodle (3)<br>Shih Tzu (1) | Thai (1)<br>Dalmatian (1)<br>Golden retrievers (1)<br>Mixed (1)<br>Poodle (3)<br>Shih Tzu (3)<br>Siberian husky (1)<br>Terrier (1) | Miniature Schnauzer (1)<br>Poodle (3)<br>Pug (1)<br>Mixed (8)<br>Sharpei (1)<br>Shih Tzu (1)<br>Spitz (2) | Beagle (1)<br>Bull dog (1)<br>Chihuahua (1)<br>Dalmatian (1)<br>Golden retrievers (1)<br>Mixed (1)<br>Poodle (4)<br>Shih Tzu (3) |
| Sex                  | M (4), F (5)                                                                                        | M (6), F (3), Fs (3)                                                                                                               | M (6), Mc (1), F (5), Fs (5)                                                                              | M (4), Mc (2), F (5), Fs (2)                                                                                                     |
| Age in years (range) | 3.56±0.95 (0.5-8)                                                                                   | 9.67±1.05 (2-17)                                                                                                                   | 7.65±0.74 (3-13)                                                                                          | 6.55±0.97 (1.17-12)                                                                                                              |

Data are presented as mean±SEM. Number in parenthesis indicates number of dogs. Abbreviation; M: male, Mc: castrated male, F: female, Fs: spayed female

The dogs in CKD groups, both stages II+III and IV, had higher plasma creatinine and BUN concentrations compared with the control and UTI groups. The significances were found in the CKD group IV compared with either the control or UTI groups ( $p < 0.05$ ).

The dogs with CKD had higher blood pressure compared with either the control or UTI groups. All blood pressures (systolic, diastolic and mean) of the dogs with CKD stage IV were significantly higher when compared with the control group ( $p < 0.05$ ). The diastolic blood pressure in the dogs with CKD stage II+III was also higher than in the control group. All blood pressures in the UTI group were not different from the control group. The diastolic blood pressure in the UTI group was significantly lower than in the CKD stage IV groups ( $p < 0.05$ ).

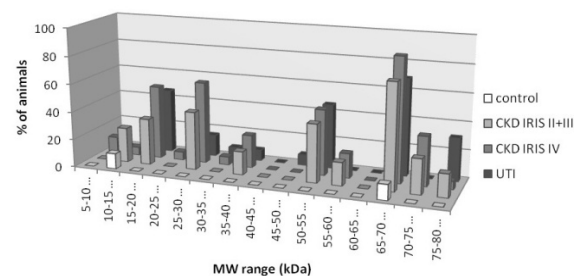
#### Urine protein profiles

Table 3 shows the urine protein concentrations found in all 4 dog groups. The UPC ratios as measured by sulphosalicylic acid were significantly higher ( $p < 0.05$ ) in dogs with CKD stages II+III and IV compared with the control group. Although the UPC ratio in the dogs with UTI was higher than normal limit, it was not statistically significant compared with the control group. The

significant increase in E-UTPC ratio was found only in the dogs with CKD stage IV compared with either the control or UTI groups ( $p < 0.05$ ). Moreover, the MMW and LMW protein creatinine ratios in the dogs with CKD stage IV were also significantly higher than the control group ( $p < 0.05$ ).

#### Distribution of urinary proteins with different molecular weight

Distributions of urinary protein with different molecular weight are shown in Fig 1. The urine proteins are distributed from molecular weight 10 kDa to 80 kDa in all groups.

**Figure 1** The SDS-PAGE electrophoresis protein distribution of dogs in 4 groups**Table 2** Packed cell volume, plasma creatinine and blood urea nitrogen concentrations and blood pressure in 4 groups of dog

| Parameters  | Control                 | CKD stage II + III       | CKD stage IV              | UTI                        |
|-------------|-------------------------|--------------------------|---------------------------|----------------------------|
| PCV (%)     | 49.1±2.1 <sup>a</sup>   | 28.8±3.3 <sup>b</sup>    | 26.9±2.2 <sup>b</sup>     | 40.8±1.3 <sup>a</sup> (10) |
| PCr (mg/dl) | 1.10±0.08 <sup>ab</sup> | 3.03±0.32 <sup>bc</sup>  | 11.57±1.72 <sup>c</sup>   | 0.99±0.05 <sup>a</sup>     |
| BUN (mg/dl) | 17.44±0.96 <sup>a</sup> | 83.92±16.22 <sup>b</sup> | 161.35±17.81 <sup>b</sup> | 15.85±1.95 <sup>a</sup>    |
| BP (mmHg)   |                         |                          |                           |                            |
| -Systolic   | 104±6 <sup>a</sup> (8)  | 128±8 <sup>ab</sup>      | 135±6 <sup>b</sup> (15)   | 122±5 <sup>ab</sup>        |
| -Diastolic  | 63±2 <sup>a</sup> (8)   | 84±8 <sup>bc</sup>       | 89±5 <sup>b</sup> (15)    | 70±4 <sup>ac</sup>         |
| -Mean       | 79±2 <sup>a</sup> (8)   | 94±7 <sup>ab</sup>       | 107±5 <sup>b</sup> (15)   | 89±4 <sup>ab</sup>         |

Data are presented as mean±SEM. Comparisons between groups in the same species were performed using one way ANOVA. Different superscripts in the same row means differ significantly ( $p < 0.05$ ). Number in parenthesis indicates the number of the animals in the study. PCV: packed cell volume, PCr: plasma creatinine concentration, BUN: blood urea nitrogen concentration, BP: blood pressure

**Table 3** Urinary protein profiles in 4 groups of dog

| Parameters              | Control                    | CKD stage II + III        | CKD stage IV             | UTI                         |
|-------------------------|----------------------------|---------------------------|--------------------------|-----------------------------|
| UPC ratio (mg/mgCr.)    | 0.085±0.027 <sup>a</sup>   | 5.806±1.565 <sup>b</sup>  | 7.263±1.993 <sup>b</sup> | 2.714±1.214 <sup>ab</sup>   |
| E-UTPC ratio (mg/mgCr.) | 0.015 ± 0.010 <sup>a</sup> | 4.632±2.093 <sup>bc</sup> | 10.23±4.573 <sup>b</sup> | 1.008 ± 0.611 <sup>ac</sup> |
| HMW proteins (mg/mgCr)  | 0                          | 0.300±0.137               | 0.936±0.443              | 0.081 ± 0.065               |
| MMW proteins (mg/mgCr)  | 0.009 ± 0.009 <sup>a</sup> | 1.542±0.513 <sup>b</sup>  | 4.685±1.979 <sup>b</sup> | 0.478± 0.295 <sup>ab</sup>  |
| LMW proteins (mg/mgCr)  | 0.006 ± 0.006 <sup>a</sup> | 2.789±1.556 <sup>ab</sup> | 5.012±2.574 <sup>b</sup> | 0.445 ± 0.250 <sup>ab</sup> |

Data are presented as mean±SEM. Comparisons between groups were performed using one way ANOVA. Different superscripts in the same row means differ significantly ( $p < 0.05$ ). UPC: urinary protein creatinine, E-UTPC: electrophoresis urinary total protein creatinine, HMW: high molecular weight, MMW: middle molecular weight, LMW: low molecular weight

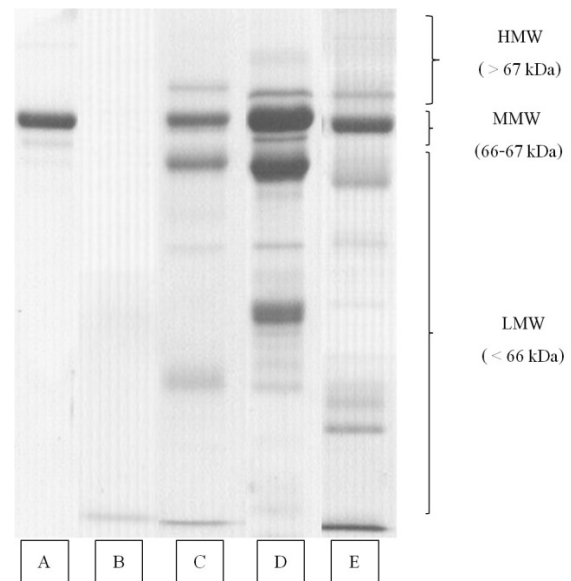
In general, urinary proteins were not recognized in the control dogs except two control dogs in which LMW of 10.06 kDa and MMW proteins of 66.4 kDa were detected. The highest percentage of protein found in the urine had molecular weight of approximately 66 kDa. Other LMW proteins in the CKD dogs that were commonly found were at 17-19, 27 and 53 kDa while the HMW proteins were at 73 and 76 kDa. The dogs with CKD stage IV had more proteins with different molecular weight than those with CKD stage II+III. The gel electrophoresis pattern in one case of each group is shown in Fig 2.

#### Relationships between urinary proteins measured by different methods

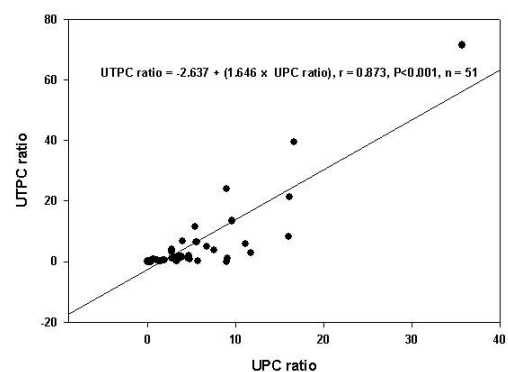
The relationship between total protein concentration as measured by sulphosalicylic acid (UPC ratio) and electrophoresis (E-UTPC) was performed using linear regression analysis. The relationship was E-UTPC ratio =  $-2.637 + (1.646 \times \text{UPC ratio})$ ,  $r = 0.873$ ,  $p < 0.001$ ,  $n = 51$  (Fig 3).

#### Relationship between severity of kidney disease, urinary protein profiles and blood pressure

The relationship between urinary protein and plasma creatinine concentrations was determined in the dogs with CKD stages II+III and IV. Only the HMW and MMW proteins were correlated with plasma creatinine concentrations ( $r = 0.486$ ,  $p < 0.01$  and  $r = 0.439$ ,  $p < 0.05$ ,  $n = 29$ ). There was a positive correlation between mean arterial blood pressure and plasma creatinine concentration ( $r = 0.397$ ,  $p < 0.05$ ,  $n = 26$ ). However, no relationship between urinary protein and blood pressure was found.



**Figure 2** SDS-PAGE electrophoretic profiles of urinary proteins in 4 groups of dogs. Lane is displayed as follows: Lane A: control molecular weight of BSA standard, Lane B: sample from control dog, Lane C: sample from dog with CKD stage II+III, Lane D: sample from dog with CKD stage IV, Lane E: sample from dog with UTI



**Figure 3** Relationship between urinary protein excretion measured by sulphosalicylic acid (UPC ratio) and electrophoresis (E-UTPC ratio)

## Discussion

The dogs with CKD and UTI were older than the control group with a variation in breed and gender. Prevalence of CKD was more common in old age due to renal dysfunction along with age. The PCV was lower significantly in the dogs with CKD stage II+III and stage IV compared with the control and UTI groups. The main reason was due to the lack of hormone erythropoietin as reported earlier in dogs with chronic renal failure (King et al., 1992). Furthermore, red blood cell lifespan may be decreased by hemolysis due to increased uremic toxin (Bonomini and Sirolli, 2003) or lipid peroxidation (Miguel et al., 1988) which was found to occur in human patients.

The dogs with CKD especially in stage IV had higher blood pressure (systolic, diastolic and mean) compared with the control group. The dogs with CKD also had significantly higher UPC ratio as measured by sulphosalicylic acid (SSA) and E-UTPC ratio from electrophoresis when compared with the control healthy group. Urinary proteins were mainly composed of MMW and LMW proteins. The positive relationship was also found between plasma creatinine concentration and either mean blood pressure or urinary proteins (HMW and MMW). Thus, the severity of renal impairment causes hypertension and proteinuria. Increased blood pressures were reported in dog with renal disease (Bartges et al., 1996; Bodey and Michell, 1996). The similar results were previously reported in dogs with renal disease which showed the weak inverse correlation between exogenous creatinine plasma clearance and either UPC ratio or systolic blood pressure (Wehner et al., 2008). They also found the weak positive correlation between UPC ratio and systolic blood pressure in dogs which was not exhibited in our study. In dogs with chronic kidney disease, hypertension and marked proteinuria were associated with significantly shorter survival times (Welner et al., 2008). Similar results were reported in dogs with spontaneous chronic renal failure which showed from Kaplan-Meier and Cox proportional hazards methods that initial high systolic blood pressure was associated with increased risk of developing a uremic crisis and of dying (Jacob et al., 2003). Therefore, higher blood pressure and proteinuria were found in the dogs with CKD stage IV compared with CKD stage II+III in this study.

Although the significant positive correlations between UPC ratio and E-UTPC ratio were found, report of negative protein detection in dog urine using different kinds of electrophoresis (SDS-AGE or HRE) was demonstrated when the UPC ratio showed borderline or proteinuria (Giori et al., 2011). Thus, UPC ratio was recommended in all cases for screening method.

Increased protein loss with renal disease was primarily due to the protein leakage passing the damage glomerular filtration membranes. The criteria was raised for glomerular renal disease if UPC ratio was  $\geq 2.0$  (Lee et al., 2005). With this criteria, 22 dogs were considered glomerular disease with UPC higher

than 2.0. Only 7 dogs had UPC less than 2.0. However, the degree of proteinuria based upon UPC ratio may not be a good indicator for diagnosing the location and type of renal disease.

By performing urinary electrophoresis, the proteins with different molecular weight could be identified. Leaking of proteins with different molecular weight may be more useful to identify the location of renal damage. The presence of middle to high molecular weight suggests the glomerular lesions while increased LMW protein may be a result of tubular lesion (Bazzi et al., 1997; Bazzi et al., 2000; Lee et al., 2005). The HMW ( $> 66$  kDa) fractional clearance and albumin (66 kDa) fractional clearance were significantly correlated to the grade of glomerular lesion while the LMW ( $< 66$  kDa) fractional clearance was moderately correlated with the tubular lesions (Biewenga and Gruys, 1986). In the present study, 5 dogs had glomerular lesion as shown by the presence of MMW proteins in the urine and 2 dogs had tubular lesion with a presence of only LMW proteins. Nineteen dogs had glomerulo-tubular lesions which exhibited both MMW and LMW while 3 dogs had no proteinuria. The results were consistent to the previous report which showed that 67% of dogs with renal disease had either HMW ( $> 76$  kDa) or MMW (66-76 kDa) and LMW ( $< 66$  kDa) which were considered as glomerulo-tubular proteinuria. However their study showed higher tubular proteinuria in which 33% of dogs had only LMW (Yalcin and Cetin, 2004). Thus, most of the dogs with CKD had pathology of both glomerulus and tubule. The tubular proteinuria may not be commonly found especially when kidney damage is in advanced stage. Some diseases such as leptospirosis caused interstitial nephritis with only LMW protein in the urine (Zaragoza et al., 2003).

The protein with molecular weight of 66 kDa was mainly albumin. In one dog of the control group, a minimal concentration of albumin was also detected although the UPC ratio was still in the normal limit and the animals did not have azotemia. The small amount of albumin could be found in the urine of normal healthy dogs (Yalcin and Cetin, 2004). In the present study, the albumin fraction was found in 24 from 29 dogs with CKD and was found in 92% of dogs with proteinuria. Higher concentration of albumin (MMW protein) was found in abundance in CKD groups and was correlated with plasma creatinine concentration. Therefore, the micro-albuminuria was used as a screening test for renal disease (Lee et al., 2005). The 74-76 kDa band may be transferrin which was detected at 76 kDa by immunoblotting from the previous study (Yalcin and Cetin, 2004). One study suggested that transferrin might be an indicator of glomerular proteinuria (Stierle et al., 1990). However, this protein was found in both CKD and UTI groups. Thus, it should not be used to indicate the glomerular disease.

The 55-65 bands may be  $\alpha_1$ - antitrypsin while the 45 kDa was heavy chain IgG or IgA (Outteridge, 1985). In the present study the protein with molecular weight of 37-39 kDa was found. This

protein may be  $\alpha$ 1-microglobulin which showed band at 30-33 kDa and was found in patients with renal failure (Yu et al., 1983; Kshirsagar and Wiggins, 1986). This protein was a good marker for tubular damage (Yu et al., 1983; Tomlinson et al., 1997). However, Yalcin and Cetin (2004) showed that the band around 30 kDa could be detected in normal dogs. The lower molecular weight proteins with bands at 10-25 kDa was reported to be related with the severity of tubular damage (Tomlinson, 1992; Bazzi et al., 1997; Bazzi et al., 2000). The protein at band 25-26 kDa may be retinol binding protein (22 kDa) or light chain IgG and IgA (25 kDa) (Outteridge, 1985). The lowest weight at 10-15 kDa may be a  $\beta$ 2-microglobulin (12 kDa). Unfortunately, the immunoblotting was not performed in this study.

The dogs with UTI also exhibited proteinuria, mostly albumin fraction, although the degree was less than the dogs with CKD stage IV. The information of proteinuria in symptomatic and asymptomatic UTI in patient was reviewed (Carter et al., 2006). They concluded that evidence of association between proteinuria and asymptomatic UTI was not found. However, symptomatic UTI is commonly associated with proteinuria and albuminuria with a poor specificity. In dogs, with experimental induced UTI with *E. coli* infection, the UPC ratio increased at 3-4 days after inoculation without renal histopathological changes (Bagley et al., 1991).

Study by Vaden and coworkers (2004) demonstrated that many dogs with pyuria did not have albuminuria and proteinuria. The 67% of urine from pyuric dogs with concurrent hematuria or bacteruria had slightly higher concentrations of urinary albumin, but not UPC ratio. Adding whole blood in the urine samples up until urine color became visible pink caused an increase in primarily albumin. Moreover, neither albumin concentration nor UPC ratios was significantly different among dogs with different degree of pyuria. Therefore, a change of urine protein was affected by hematuria when the degree of UTI was in advanced stage. The electrophoretic pattern of samples obtained by adding serum/hemolysate into the urine and in samples of post-renal hematuria were similar (Bianchi-Bisio et al., 1991). Thus, the presence of hematuria should be considered extensively when measuring the protein fractions in the urine. In the present study, hematuria with pyuria was found in 5 cases while bacteruria was found in 9 cases from the total of 13 cases. In addition to the albumin fraction, the proteins with various molecular weights were found although the degree of protein loss was less than the CKD groups. Study cases of urinary tract infection with cystitis or pyelonephritis showed that 86% of children with cystitis had small amount of albumin with no LMW protein (Bianchi-Bisio et al., 1991). If the hematuria was presented, the high molecular weight proteins from 60 to 500 kDa were also found. Study in acute pyelonephritis which may involve the impaired tubular reabsorption of filtered protein, the LMW proteins (10-67 kDa) were demonstrated in 70% of cases and were correlated with the severity of infection. Moreover, treating the patients could

reduce the LMW proteins. The  $\alpha$ 1-microglobulin excretion, which is the glycoprotein (30-33 kDa) was above normal range in all cases of pyelonephritis. High urinary  $\alpha$ 1-microglobulin creatinine ratio was also found in children with acute pyelonephritis but not in cystitis (Everaert et al., 1998). Thus,  $\alpha$ 1-microglobulin could be used to distinguish between acute pyelonephritis and cystitis. The elevated  $\beta$ 2-microglobulin excretion and clearance were reported in patients with upper urinary tract infection but not cystitis (Schardijn et al., 1979; Mengoli et al., 1982). Another study reported the increased urinary albumin,  $\alpha$ 1-microglobulin,  $\beta$ 2-microglobulin, retinol binding protein and an enzyme N-acetyl-beta-glucosaminidase in acute pyelonephritis but not in cystitis and asymptomatic bacteriuria (Sandberg et al., 1985). Thus, the upper UTI may be associated with tubular protein pattern which was not presented in animals with lower UTI. Furthermore, they found that increased tubular proteins might be a response to fever without renal involvement. Our study showed the presence of LMW, MMW and protein molecular weight higher than albumin, but less than 98 kDa. Differentiating the location of UTI using urinary protein of varying molecular weight may not be possible. Moreover, the pattern of protein was similar to the dogs with CKD.

In conclusion, the proteinuria was found in dogs with both CKD and UTI. The degree of proteinuria was remarkable in CKD stage IV. However, the degree and pattern of urinary protein with different molecular weight could not be used to distinguish between CKD and UTI in dogs.

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