

INFLUENCE OF RENAL ULTRASONOGRAPHIC FINDINGS ON GFR DURING CHRONIC UNILATERAL URETERAL OBSTRUCTION IN DOGS

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ABSTRACT

Renal sonographic findings can now be implicated for prediction of changes in Glomerular filtration rate, and estimation of the degree of renal dysfunction in pets. This was successfully determined during a 20-day chronic unilateral ureteral obstruction in experimental dogs. GFR was quantitatively measured following a single intravenous bolus of inulin. The total clearance of the marker was calculated from the decrease in serum concentrations using a two-compartment model. Changes in renal architecture (i.e. length, width, depth, cortex, pelvis and ureteral dilation) were simultaneously assessed using B-mode ultrasonography. The results showed significant changes in GFR and renal architecture. Statistical analysis revealed minimum intragroup variance in renal length, width and depth. Linear regression analysis indicated a highly significant average correlation coefficient (r) with low standard error of estimates (SEE) and greater predictability between GFR and left renal dimensions of length ($r=-0.9454$, SEE= 2.64), width ($r= -0.8632$, SEE= 1.69), depth ($r=-0.9461$, SEE= 2.11), pelvis ($r=-0.9035$, SEE= 3.91) and left ureter ($r=-0.9714$, SEE= 2.07). The prediction error was about 9-12%. Conclusively, ultrasonographic changes in renal dimensions, particularly renal length, width and depth may successfully be used in dogs for prediction of changes in GFR, without the need to perform complicated laboratory procedures in future.

Keywords: ureteral obstruction; GFR; ultrasonography; dogs.

INTRODUCTION

Urine outflow obstruction, due to calculi, inflammatory disease, neoplasia, stricture or accidental ureteral ligation during ovariectomy, is the common presenting complaint in face of a currently increasing frequency of urinary tract disorders in pets (Brown, 2003). Undiagnosed, neglected, or untreated cases can lead to renal hydronephrosis, and hence, progression of an acute reversible condition to a chronic irreversible malady.

In unilateral ureteral obstruction, compensatory renal responses mask the injury and its effect on the renal patient during the first phase of renal damage. The animal is not azotemic but may only have some reduced urine concentrating ability (Klahr *et al.*, 1988; Watson *et al.*, 2002a; Brown, 2003). Furthermore, as reported by Braun and Lefebvre (2008), and Brown (2003), the large renal functional reserve ensures maximal compensation, so that clinical signs and routine laboratory indices generally do not indicate severity of the renal disease, until 75% of renal mass is destroyed. The results of this experimental study correlated exactly well with all these findings mentioned above. Hence, this necessitates the implementation of additional and precise diagnostic aids for early assessment of the degree of renal damage and successful monitoring for follow-up patients.

The glomerular filtration rate (GFR) is currently considered the best parameter for direct quantitative assessment of overall renal function (Brown, 2003; Laroute *et al.*, 1999). The single-injection clearance of Inulin is an attractive, cheap, precise and effective alternative to the other more complicated and expensive methods; however the set-back lies in the method being time-consuming and hence, less commonly applied in routine veterinary practice, making the clinician ultimately rely upon routine laboratory indices. For qualitative assessment of renal architecture, B-mode ultrasonography is currently one of the most sensitive, least-invasive diagnostic modality, providing additional benefits of direct visualization of normal structure of internal organs, characterization and location of lesions and collection of biopsy samples. (DiBartola, 2005; Hager *et al.*, 1985; Konde *et al.*, 1984). Recent studies conducted in humans negate the misconception that sonography is unable to provide information on renal function. It has now been proved by some scientists that renal sonography provides useful information about renal function. Similar data in animals has not yet been researched.

The present study was thus conducted to evaluate the architectural and functional changes in renal tissue in experimental models mimicking unilateral obstructive uropathy; and thus to ascertain the best correlation and

degree of predictability of GFR values using gray-scale sonographic changes observed in the affected kidney, for futuristic convenience and success in monitoring progressive renal disease in dogs.

MATERIALS AND METHODS

Dogs: Twenty-four healthy mongrel dogs of either sex, aged from 6 months to 6 years and weighing 6 to 11 kg were selected in this study. The study protocol was approved by the Animal Ethics Committee of the Nanjing Police Dog Research Institute of Public Security Ministry. After acclimatization and complete health check-up, jugular catheterization was performed using a 16 gauge indwelling catheter, for convenient blood sampling (Bright, 2003). Just a day before the launching of the experimental study, the left ureter was ligated in each dog through a midline laparotomy, to create experimental models of obstructive uropathy.

Test substances: For the direct quantitative assessment of renal function, Inulin (50g equivalent to 173.7g) was purchased from China National Medicines Corporation Ltd., Beijing, and used as test marker.

For each kinetic study, Inulin was reconstituted aseptically with 0.9% saline solution to obtain a final concentration of 250mg. mL⁻¹ (Miyamoto, K, 1998; Laroute *et al.*, 1999).

Real-time ultrasonographic scans of all dogs were performed after the kinetic studies, using a small animal ultrasound unit granted by Keen Trade Co., Wuxi, China.

Experimental design: The dogs were randomly assigned to four groups of six animals each. In all experimental dogs, direct measurement of renal function was carried out to quantitate the degree of damage, using Inulin plasma clearance assays.

For precise functional assessment of the various stages of renal dysfunction, each kinetic study was carried out on 0, 1, 3, 5, 7, 11, 15 and 20 days after the left ureteral ligation. Day 0 dogs served as control (i.e. before ureteral ligation).

Prior to the clearance studies, food was withheld for at least 12 hours, however, the dogs were provided free access to water before and during the procedure (Haller *et al.*, 1998; Laroute *et al.*, 1999).

Inulin clearance: On each day of the kinetic study, Inulin was administered, as a single intravenous injection, at a dose rate of 100 mg/kg, via an indwelling catheter placed in the cephalic vein of either limb (Laroute *et al.*, 1999). The catheter was flushed with 4 mL of saline solution (0.9% NaCl) after the injection. The total duration of administration of Inulin was 20 seconds. Blood samples were obtained from the jugular vein via the indwelling jugular catheter. For Inulin assays, blood

samples were obtained at time 0 (i.e. before injection), and at one, three, 10, 20, 40, 80 and 120 min after injection. The samples were allowed to clot and were centrifuged at 3000 rpm for 10-15 min; the serum thus collected was frozen at -20 °C until analysis.

Laboratory analysis: The concentration of Inulin was measured with an optimized colorimetric method.

Inulin assay: The concentration of Inulin in each serum test sample was determined by a chemical method using anthrone reagent (Wright and Gann, 1966; Jung *et al.*, 1990) and spectrophotometric readings were taken at an absorbance value of 636 nm.

The concentration profile following bolus injection of Inulin showed a biphasic behavior pattern: the initial rapid decrease in the plasma level was followed by a phase of gradual decline. Such a time curve can be described by the sum of two exponential functions (Jung *et al.*, 1992). For this reason, a two-compartment model was considered to suitably fit the curve, and used to calculate the glomerular filtration rate with the aid of the computer software 3P97, using the formula (Sapirstein *et al.*, 1955):

$$\text{GFR} = \frac{\text{dose}}{A/a + B/b}$$

Where

GFR	= glomerular filtration rate
A	= intercept of the rapid phase
B	= intercept of the terminal phase
a	= first-order rate constant of the Rapid phase
b	= first-order rate constant of the terminal phase.

Ultrasonography: B-mode ultrasound assessment was performed on the same day as the kinetic studies, in order to assess the qualitative changes in renal tissue, during progression of the renal dysfunction in experimental dogs.

After appropriate patient preparation (i.e. hair clipping from ventral abdomen, thorough cleaning of the skin with surgical spirit, and application of acoustic gel) (Mattoon, *et al.*, 2002), both right and left kidneys were scanned in standard sagittal and transverse planes (Nyland *et al.*, 2002; Holt, 2008), using a 5.0 MHz convex-array curvilinear transducer. Length, width, depth, thickness of cortex, distance of pelves of each kidney and opening of the left ureter were recorded.

Statistical Analysis: The results were analyzed by application of ANOVA for calculation of the means. Correlations of GFR with left renal dimensions were assessed through linear regression analysis. All statistical analyses were performed with SAS statistical software (SAS Institute Inc., USA).

RESULTS

Changes in Renal Function assessed through calculated Mean GFR values: In the experimental dogs, the GFR value decreased gradually with progression of renal disease after ureteral ligation. Hence, from a normal recorded mean value of 2.585 ± 0.225 mL. min⁻¹. kg⁻¹ on Day 0, GFR showed a highly significant decrease on Days 3, 8 and 11 ($P < 0.01$); the least recorded mean value of GFR was 0.754 ± 0.063 mL. min⁻¹. kg⁻¹ on Day 20 ($P < 0.05$)- **Table 1**.

Changes in Renal Architecture assessed through Ultrasonographic findings: During the 20-day ureteral ligation, left renal length, width and depth showed enormous increases (**Table 1**). When compared with Day 0, the changes in left renal length, width and depth on each day were highly significant ($P < 0.01$).

Left renal pelvis, likewise, showed significant increase in size from 7.37 ± 0.32 on Day 0, to 26.14 ± 3.40 mm on Day 20 (**Fig. 1C**). When compared with Day 0, the changes were significant ($P < 0.05$ on Days 2 and 3, and $P < 0.01$ on Days 4, 6, 8, 10, 12, 14, 17 and 20, respectively). Similarly, when compared with Day 0 findings, a significant decrease in left renal cortical thickness was observed on Days 10 and 12 ($P < 0.05$); thereafter, the decrease in cortical thickness due to pressure atrophy of renal tissue, was highly significant i.e. $P < 0.01$ on Days 14, 17 and 20, respectively (**Fig. 1E**).

Changes in the opening of the left ureter were most conspicuous and significant on each day, ranging from 1.00 ± 1.00 mm on Day 0 to 23.28 ± 2.44 mm on Day 20 ($P < 0.01$) – (**Table 1 and Fig. 1**).

The Measured Values of GFR and Renal Dimensions in Experimental Dogs: The average values of the

different variables (GFR and renal length, width, depth, pelvis, cortex and ureter) are shown in Table 2. The relative intra-group variance expressed as CV% was least for renal width, length and depth, respectively, as compared to GFR (~7 vs 39%). The average co-efficient of variation (CV%) was increasingly high for renal cortex, pelvis and ureter (21, 41 and 53%, respectively), suggesting higher variance.

Linear Regression Analysis and Calculation of Correlation Coefficient between GFR and Left Renal Dimensions: Table 3 summarizes the results of linear regression analysis; the correlation coefficient (r) and standard error of estimate (SEE) are shown. GFR is found to be highly correlated with ultrasonographic changes recorded in the left kidney, post-ligation, with a high value of r and low SEE. The correlation coefficient (r) of GFR with left renal length, and the standard error of estimate (SEE), respectively, are -0.9454 and 2.64. GFR also exhibits a high correlation with left kidney width, depth, pelvis and ureteral opening, the values of r and SEE respectively, being -0.8632 and 1.69 (GFR and width), -0.9461 and 2.11 (GFR and depth), -0.9035 and 3.91 (GFR and left renal pelvis), and -0.9714 and 2.07 (GFR and left ureteral opening). Predictability was greatest between GFR and length, depth and left ureteral size ($P < 0.0001$), moderately between GFR and width and pelvis ($P < 0.01$) and lesser between GFR and cortex ($P < 0.05$). The prediction error was about 9-12%. In comparison with other variables, smaller intra-group variability (CV%) between renal dimensions of length, width and depth, smaller SEE and high correlation of the variables with GFR, enhanced the overall accuracy in predictability of GFR, through the regression equation (**Fig. 2**).

Table 1. Quantitative Changes in GFR and Left Renal Length, Width, Depth, Pelvis, Cortex and Left Ureteral Opening During the 20-Day Experimental Period

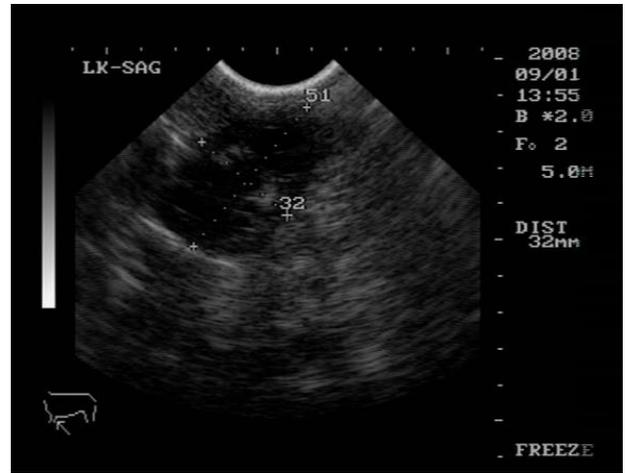
Day	GFR (ml/min/kg)	Left Renal Length (mm)	Left Renal Width (mm)	Left Renal Depth (mm)	Left Renal Pelvis (mm)	Left Renal Cortex (mm)	Left Upper Ureteral Opening (mm)
0	2.58±0.23	51.58±0.76	27.87±0.58	25.87±0.43	7.37±0.18	5.52±0.22	1.17±0.08
1	1.96±0.11 ^a	59.92±0.93 ^a	34.58±0.64 ^a	31.75±0.55 ^a	9.87±0.37 ^a	5.35±0.22 ^a	6.62±0.29 ^a
3	1.48±0.09 ^a	61.58±0.82 ^a	34.16±0.99 ^a	31.87±0.49 ^a	11.79±0.32 ^a	5.17±0.27 ^a	9.33±0.27 ^a
5	1.324±0.09 ^a	61.37±0.96 ^a	34.83±0.65 ^a	34.54±0.43 ^a	15.67±0.39 ^a	4.61±0.21 ^a	13.83±0.59 ^a
8	1.32±0.09 ^a	63.25±0.85 ^a	33.08±0.60 ^a	34.21±0.49 ^a	16.25±0.43 ^a	3.82±0.23 ^a	14.67±0.52 ^a
11	1.21±0.12 ^a	66.50±0.76 ^a	34.37±0.41 ^a	38.54±0.47 ^a	22.12±0.55 ^a	3.48±0.18 ^a	17.12±0.49 ^a
15	1.37±0.15 ^a	68.46±1.06 ^a	35.92±0.82 ^a	39.17±0.63 ^a	24.08±0.78 ^a	4.0±0.24 ^a	17.83±0.88 ^a
20	0.75±0.13 ^a	67.25±1.02 ^a	36.33±0.80 ^a	40.29±0.87 ^a	26.17±1.00 ^a	3.74±0.22 ^a	23.29±0.92 ^a

N.B. a: $P < 0.001$, means highly significant, c: $P < 0.05$, means significant;

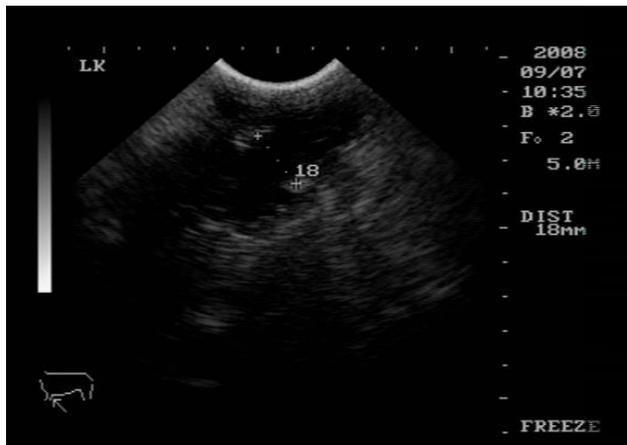
b: $P < 0.01$, means moderate significance; NS: $P > 0.05$, means non-significant.



(A) Sagittal Scan of Left Kidney-Day 1- length x depth=51 x 27 mm



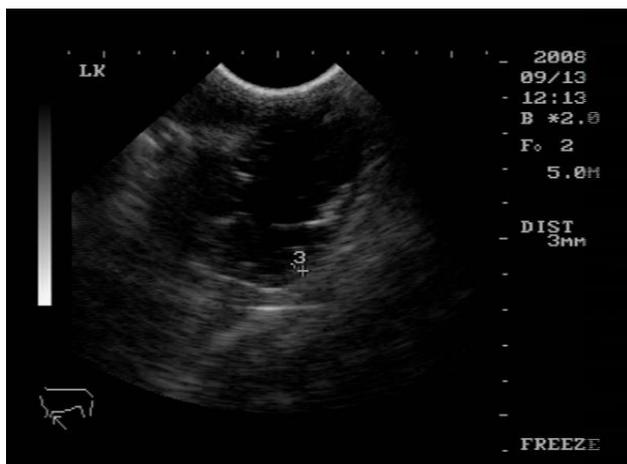
(B) Sagittal Scan of Left Kidney Day 8- length x depth=51 x 32mm



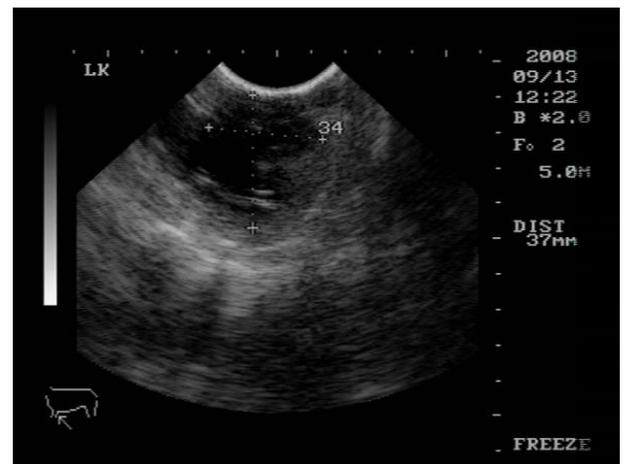
(C) Sagittal Scan of Left Kidney-Day 14: Interpelvis distance:18mm



(D) Transverse Scan of Left Kidney- Day 14: width x depth= 28 x 28 mm.



(E) Sagittal Scan of Left Kidney-Day 20- cortex thickness: 3 mm



(F) Transverse Scan of Left Kidney-Day 20: width x depth= 34 x 37 mm .

Fig. 1 Ultrasound Scans showing Prominent Changes in Renal Architecture

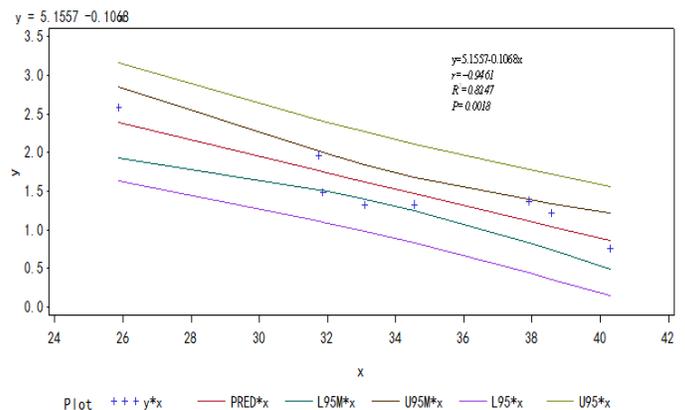
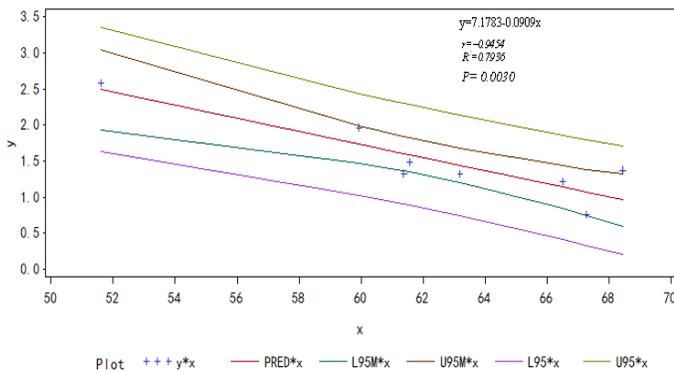
Table 2. Average Coefficient of Variation of GFR and Renal Dimensions

Variables	Average Mean \pm S.D	Average Coefficient of Variation (CV/ %)
GFR	1.462 \pm 0.20	39.086
Length	62.49 \pm 1.90	8.608
Width	33.89 \pm 0.93	7.773
Depth	34.53 \pm 1.69	13.870
Pelvis	16.67 \pm 2.44	41.349
Cortex	4.46 \pm 0.34	21.775
Ureter	12.98 \pm 2.48	53.979

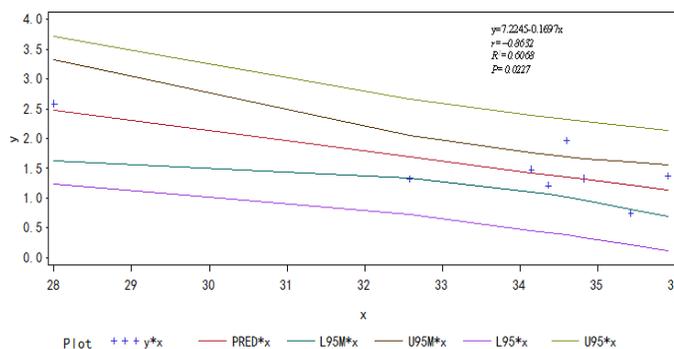
Table 3. Results of Linear Regression showing high Correlation of GFR with Renal Dimensions, with high predictability

Variables (x)	n	Formula for the Calculation of GFR (y) in relation with renal dimensions	Correlation coefficient (r)	P values	SEE	SEE %
Length	24	y= 7.1783-0.0909x	-0.9454 ^a	0.0030	2.638	9.21
Width	24	y=7.2245-0.1697x	-0.8632 ^b	0.0227	1.697	10.32
Depth	24	y=5.1557-0.1068x	-0.9461 ^a	0.0018	2.108	9.71
Pelvis	24	y=2.6282-0.0678x	-0.9035 ^b	0.0075	3.910	12.25
Cortex	24	y=-0.3411+0.4123x	0.8029 ^c	0.0393	0.717	11.62
Ureter	24	y=2.4719-0.0758x	-0.9714 ^a	0.0001	2.073	9.17

N.B. a: $P < 0.001$, means highly significant, b: $P < 0.01$, means moderate significance; c: $P < 0.05$, means significant; NS: $P > 0.05$, means non-significant.



A- Relationship between GFR and Left Renal Length



BB- Relationship between GFR and Left Renal Width

C- Relationship between GFR and Left Renal Depth

Fig. 2. Linear Regression interrelationship between GFR and Left Renal Dimensions

DISCUSSION

The objectives of this study were aimed towards quantitative and qualitative determination of the degree of renal dysfunction produced as a result of experimental unilateral ureteral obstruction; and the interrelationship and prediction of changes in GFR with ultrasonographically observed changes in the dimensions of the affected kidney, for futuristic convenience in GFR

calculation in clinically ill renal patients.

In clinical situations, obstructive uropathy in pets can lead to profound hydronephrosis, which if left untreated or undiagnosed, can cause permanent renal damage and chronic renal failure, as a result of pressure atrophy of the kidney tissue (Kahn *et al.*, 2005; Vegad and Katiyar, 1998). Although in early stages of renal disease in dogs, the routinely-used indices do not indicate an ongoing disease process, however, the direct functional markers and ultrasonographic assessment enable the clinician to detect minute changes in GFR and renal architecture, earlier in the course of a progressive renal disorder, thus holding importance as regards treatment protocols and monitoring of follow-up patients. However, since the routine calculation of GFR is a time-consuming procedure and alternative formulae used in human medicine for GFR calculation are not popular in veterinary practice, hence we have predicted significant correlation of the functional GFR changes with morphological changes in affected renal tissues, which can successfully be applied to routine veterinary practice in future.

For determination of the glomerular filtration rate, Inulin is still the accepted “gold standard” amongst all indicator substances; toxicity for this compound is very low (Brown, 1994), and no side effects were observed in this study, either (Haller *et al.*, 1998). Furthermore, the strategy of determining the total plasma clearance of the test marker after a single bolus injection with repeated blood sampling over a limited period of time (Laroute *et al.*, 1999), proved a time-saving, precise and better alternative to continuous infusion or urine sampling techniques. One striking result of this study was that Inulin concentration could successfully be determined by application of the two-compartment model (Haller *et al.*, 1998; Laroute *et al.*, 1999).

Immediately after left ureteral ligation, a highly significant decrease in GFR was evidenced on Days 3, 5, 8 and 11 ($P < 0.01$). With progression of time, most experimental dogs showed a reduced renal function, as compared to the normal dogs ($P < 0.05$ on Days 15 and 20). In some dogs, however, there were observable fluctuations in the GFR value with a tendency to rebound towards improvement at various times; this could be attributed to the early stage of renal disease (because the experimental study lasted less than three months); early renal disease usually does not cause loss of the whole nephron, and hence the GFR can be observed to be close to normal at various times, because of the hyperfiltration of other nephrons and other compensatory mechanisms of the contralateral kidney. This observation of GFR returning towards a normal value, despite slight insignificant proteinuria, was hence not surprising (Grauer and DiBartola, 1995). However, it was simultaneously observed that the compensatory changes in the body occurring alongside the inflammatory process

were not consistent or supportive to repair the hydronephrotic lesion completely. As a result, GFR value decreased consistently (Klahr, *et al.*, 1988), with an overall aggravated compromising condition of the experimental animals, towards the end of the experimental study.

Qualitative assessment of renal dysfunction through sonograms obtained post-ligation, revealed that longer the duration of urinary obstruction, greater is the degree of dilatation (and hence, pressure atrophy) of the renal pelvis (Canpolat *et al.*, 1996; Cartee, *et al.*, 1980; D’Anjou *et al.*, 2011). After two weeks of the experimental study, the size of the renal pelvis increased to about 3.54 times its normal size; the increase in renal length and width were approximately 1.3 times the normal value, and renal depth increased to about 1.5 times the normal. After about 3 weeks post-operatively, the left ureter showed 23 times increase in its size from normal (Table 1); in some dogs such immense increase in size made it a bit difficult to discriminate between the pressure-atrophied, enlarged pelvis, from the hydroureter.

In humans, the presence of a close correlation between kidney size and function is reported to be helpful in the diagnosis of renal diseases (Adibi *et al.*, 2007; Kariyanna *et al.*, 2004; Paleologo *et al.*, 2007; Widjaja *et al.*, 2010). For our experimental study in canines, the relationship between GFR and affected kidney morphology investigated through simple linear regression analysis yielded a highly significant (inverse) correlation, low standard error of estimate and greater predictability between GFR and left renal length, depth and ureteral size (Table 2). Furthermore, the results in Table 2 showed a significantly smaller coefficient of variation for renal length, width and depth, as compared to the GFR, thus emphasizing smaller group variability and greater predictability. The group variability of cortex, pelvis and ureteral size was higher than other renal parameters, and hence we infer that these parameters may not be considered as reliable for prediction of GFR, as renal length, width and depth. It can however, be hypothesized that sonographic measurements proved to be highly specific, while GFR measurements showed high sensitivity and lower specificity. Additionally, in contrast to studies in humans emphasizing greater predictability of GFR solely through renal length, we propose that sonographic renal length, width and depth measurements in canine patients may prove to be highly prognostic of GFR changes in renal disease.

It can thus be concluded that despite immense structural damage to the renal architecture, in cases of prolonged urine outflow obstruction, the kidneys compromise for the functional changes by compensatory responses which minimize the total loss in GFR. Furthermore, sonographic measurements of affected kidneys not only prove as a non-invasive method, rather, renal length, width and depth correlate well with GFR

values in canines. Hence, they can importantly be employed, successfully and conveniently, in routine veterinary clinical settings in future, for precise measurement of renal function, assessment of the degree of renal dysfunction in affected animals, and as an alternative to expensive, complicated and time-consuming methods.

(Total words: 2, 715).

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Ethical Standards: The authors hereby declare that the experiments comply with the current laws of the country in which they were performed and International Standards. Furthermore, animal use ethics were adopted as permitted by the Animal Ethics Committee of the Nanjing Police Dog Research Institute of Public Security Ministry, P. R. China and as guided under International legislation.

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