CORRELATION BETWEEN CHANGES IN SONOGRAPHIC, HISTOPATHOLOGICAL AND LABORATORY INDICES IN DOGS WITH EXPERIMENTAL POST-RENAL OBSTRUCTION

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ABSTRACT

Unilateral post-obstructive azotemia clinically manifests as a progressive disease syndrome producing diverse irreversible derangements in renal dynamics. Renal dysfunction was assessed through experimental left ureteral ligation in dogs for 20 days. Sonographic changes in renal tissue, and hematological, biochemical, histopathological and urinalysis indices were evaluated on Day 0 and 1, 3, 5, 8, 11, 15 and 20 days after surgery. Sonography revealed significant increase in renal length, width and depth (P<0.05) post-surgery. Leukograms indicated inflammatory lymphocytosis and leucocytosis with a left shift. Hemograms depicted severe, regenerative, normocytic, normochromic anemia and a strong negative correlation with sonographic length, width and depth; thrombograms revealed significant thrombocytopenia on Day 11 (P<0.01). Biochemical profiles yielded significant hypoproteinemia, hypoalbuminemia, hypernatremia and hyperchloremic acidosis (P<0.01). Total protein concentrations showed moderate inverse correlation, while sodium and albumin concentrations, respectively, showed strong, highly significant correlations with sonographic length and depth. Decreased urine concentrating ability was evidenced by decreased urine specific gravity (USG< 1.030, P<0.05) on Days 2, 6, 8, 10 and 12, and a highly significant correlation with sonographic length, width and depth.Tissue sections depicted Grade 4 (61-80%) damage, exhibiting tubular atrophy with subsequent thinning of the renal parenchyma, and a strong correlation with sonographic length (P<0.01) and depth (P<0.01). Using the formulae, $Y_{HP} = -11.93 + 0.072X_L$ and $Y_{HP} = -11.93 + 0.267X_D$, severity of histopathological changes can be depicted from an increase in sonographic length and depth, respectively. Conclusively, B-mode sonography is a sensitive modality since architectural changes signify and correlate with derangements in GFR and cellular and microscopic changes, manifested systemically.

Keywords: Ultrasonography; hematology; serum biochemistry; urinalysis; histopathology.

INTRODUCTION

Unilateral urine outflow obstruction leads to post-renal azotemia, which causes functional atrophy of the renal tissue clinically manifested as the hydronephrosis syndrome. Common causes include ureteral or urethral blockage by calculi, chronic inflammation and neoplasia of the renal pelvis, ureter or urinary bladder, misplaced sutures (during an ovariohysterectomy surgery), foreign bodies in the ureters and uterine stump granulomas.

Post-obstructive renal dysfunction microscopically, involves a progressive loss of the filtration function of the affected kidney, accompanied by systemic hemodynamic derangements. Progressive failure of filtration function is evidenced by decrease in glomerular filtration rate (GFR), azotemia (characterized by the accumulation of nitrogenous waste in blood), disordered electrolyte metabolism and changes in the body fluid status.

Protracted urine outflow obstruction for 4 weeks or longer results in a permanent loss of function of the associated kidney (Christie and Bjorling, 1993). The pathogenesis involves a perpetual pressure atrophy of the renal parenchyma due to increased hydrostatic pressure from the obstructed ureter or urethra, to the renal pelvis, renal tubular system and Bowman’s space; the ultimate result of which, is a progressive filtration failure manifested clinically as a decline in GFR due to compensation failure in the affected kidney. In unilateral obstruction, however, the contralateral kidney undergoes compensatory hypertrophy and generates responses which mask the renal insult. As a result, the animal manifests only mildly reduced urine concentrating ability (Watson et al., 2002a; Brown, 2003; Klahr et al., 1988) with no apparent azotemia, till about 75% reduction in renal mass (Braun and Lefebvre, 2008; Brown, 2003).

In this project, the correlation between sonographic findings and hematologic, biochemical, histopathological and urinary derangements were studied during various stages of post-obstructive uropathy. The objectives were to emphasize the importance of diagnostic aids for timely and effective remedial efforts in order to prevent advancement of renal dysfunction in
affected patients. Furthermore, a correlation between sonographic and laboratory findings was sought. Additionally, severity of histopathological changes was categorically graded through the correlation analysis.

**MATERIALS AND METHODS**

A. **Preparation of Dogs**: This study was conducted at Nanjing Agricultural University, after approval by the Animal Ethics Committee of the Nanjing Police Dog Research Institute of Public Security Ministry (reference number: SYXK(SU)2007-0001). This was an extensive study involving kinetic studies in dogs. However, this paper covers sonographic and laboratory data assessment, only.

After careful selection, thorough health check-up and acclimatization, 24 healthy mongrel dogs (average weight between 6 to 11 kg) were randomly divided into six equal groups, A, B, C, D, E and F, comprising 4 animals each. In all groups, through surgical exposure, the jugular vein of each dog was catheterized using a 16-Gauge indwelling catheter (Bright, 2003), to ensure convenient blood sampling. Additionally, the left ureter in each dog was ligated via a midline laparotomy, just a day before launching the study, in order to construct experimental models of unilateral urine outflow obstruction.

For functional assessment of the various stages of renal dysfunction, each analysis was performed on Day 0 (Control Group), Day 1 (Group A), Day 3 (Group B), Day 5 (Group C), Day 7 (Group D), Day 15 (Group E) and Day 20 (Group F), after the left ureteral ligation. In Day 0 dogs (Control Group), all studies were performed in the healthy animals, i.e. before ureteral ligation.

B. **Collection of samples for hematology, blood chemistry and urinalysis**: On each day of the experimental study, blood samples were collected from the jugular vein via the indwelling jugular catheter. 0.5 mL blood samples were collected in separate microcuvettes, and haematological studies were carried out with the help of hematology analyser, HB-7021Sinnowa®.

For biochemical examination, maximum amount of serum was withdrawn from the blood samples, and the serum was stored at −20°C till analysis via Blood Chemistry Unit, Selectra-E Plus 2005, Vita Lab. Co.

Urinalysis examination was performed via urine dipsticks (KruLab®, Denmark) on about 5 mL freshly voided urine samples collected every morning, for a duration of 20-days.

C. **Sonographic Scanning**: A 5.0 MHz or 7.5 MHz convex-array curvilinear transducer was used to scan standard sagittal and transverse planes of both kidneys (Holt, 2008; Nyland et al., 2002). Sonography was performed on the same day after laboratory analysis, in order to assess the qualitative changes in renal tissue, during simultaneous progression of the renal dysfunction. Changes in length, width, depth, thickness of cortex, distance of pelves of each kidney and opening of the left ureter were recorded.

D. **Histopathological Evaluation of Renal Tissue**: Sections of left kidney tissue, prepared from biopsy samples obtained at various times during the experimental period, viz. Days 1, 3, 5, 7, 15 and 20 post-ureteral obstruction. Sections were stained using hematoxylin and eosin staining technique.

Depending on the severity of renal insult, histopathological changes in affected renal tissue were scored as grade 0 (0% damage, Control Group- pre-operatively), grade 1 (1-10 % damage), grade 2 (20-40% damage), grade 3 (41-60% damage), grade 4 (61-80% damage), and grade 5 (> 80% damage), respectively.

**Statistical analysis**: The results were analyzed by application of ANOVA for calculation of the means using SAS statistical software (SAS Institute Inc., USA).

Correlation analysis was performed to assess degree of correlation between sonographic and respective laboratory parameters.

Rank correlation analysis was performed to correlate sonographic changes to histopathological changes. Furthermore, linear regression was used to predict severity of histopathological changes from sonographic length and depth.

**RESULTS**

**Changes in Renal Architecture assessed through Ultrasonographic findings**: The changes in left renal length, width and depth on each day during the 20-day ureteral ligation, were highly significant (P<0.01), when compared with Control (Day 0)- Chart 1.

Left renal pelvis, likewise, showed significant increase in size from 7.37± 0.32 on Day 0, to 26.17±3.40 mm on Day 20. When compared with Day 0, the changes were highly significant (P<0.01).

Left renal cortex significantly (P<0.01) receded in thickness during the experimental period due to pressure atrophy of renal tissue.

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) – (Chart 1).

**Changes in Hematological Profile**: Hemograms depicted a severe regenerative normocytic (normal MCV), normochromic (normal MCHC) anemia evidenced by a highly significant decrease in RBC counts, haemoglobin concentration and haematocrit throughout the experimental period (P<0.01). Day 1 leukograms
indicated marked leucocytosis due to neutrophilia with a regenerative left shift; marked granulocytosis, and moderate lymphocytosis (P<0.01) were also simultaneously observed on Day 1 after ureteral ligation. Additionally, a significant thrombocytopenia was seen on Day 11 (P<0.05).

Changes in Biochemical Profile: The biochemical profile was clearly indicative of a highly significant hypoproteinemia (Day 11, P<0.01) and hypoalbuminemia (Days 11 and 15, P<0.01). Uric acid indices significantly spiked by Day 20 (P<0.01), whilst hyperchloremic acidosis (Day 20, P<0.01), and recurrent hypernatremia (Days 11 and 20, P<0.01) were the simultaneous, most conspicuous findings, suggestive of the progressive devastating pattern of the renal insult.

Changes observed on Urinalysis: Since urinalysis was performed every morning on freshly voided urine samples, from dogs of various groups, hence, a significant decrease in urine specific gravity (USG<1.030) was observed on Days 2, 6, 8, 10 and 12 (P<0.05). This was a manifestation of the on-going nephron loss and subsequent decreased urine concentrating ability in the experimental animals.

Changes observed on Histopathological Examination: Depending on the severity of renal insult, histopathological changes in affected renal tissue were scored as grade 0 (0% damage, pre-operatively), grade 1 (1-10% damage), grade 2 (20-40% damage), grade 3 (41-60% damage), grade 4 (61-80% damage), and grade 5 (>80% damage), respectively.

Rank correlation analysis revealed a highly significant difference between renal length (P<0.001) and renal depth (P<0.000), when compared with renal histopathological changes.

Correlation Analysis: Correlation analysis revealed a highly significant inverse correlation of RBC no., Hemoglobin (HgB) and hematocrit (HCT) with renal length, width and depth, respectively.

Changes in urine specific gravity showed strong correlation to ultrasonographic parameters of depth, length and depth, respectively.

Correlation data are summarized in Table 2.
Chart 1. Sonographic Changes Recorded in Left Kidney Post-Ureteral Ligation

Chart 2. Changes in the Hematological Indices of Experimental Animals

NB: * Indicates that correlation is significant, \( P < 0.05 \) level; ** Indicates that correlation is highly significant, \( P < 0.01 \) level.

RBCs: \( (n \times 10^{12} \text{ L}^{-1}) \); HGB: (g. L\(^{-1}\)); HCT: (percentage %); Lymphocytes, Granulocytes, WBCs and PLT: \( (n \times 10^9 \text{ L}^{-1}) \)
Figure 1. Histopathological changes in left renal tissue during the 20-day ureteral ligation. A, Renal histopathology (HE, 100x) Grade 1 (1-10 %) damage - Renal abscess, 3rd day post-ligation; the tubules characterize haemorrhage and degeneration (arrows). B, Grade 3 (41-60%) damage - Mesangial Cell Hyperplasia (HE, 100x), 7th Day Post- Ligation. C, Left renal histopathology - (HE,100x), Interstitial edema (arrow) - 14th Day Post-Ligation. D, Grade 4 (61-80%) damage, Apoptotic tubules (arrow) with interstitial oedema (HE,100x) - 14th Day Post- Ligation

Table 1. Changes in Biochemical Indices and Urine specific gravity of Experimental Animals.

<table>
<thead>
<tr>
<th>Parameters/ Days</th>
<th>TP</th>
<th>ALB</th>
<th>UA</th>
<th>Na</th>
<th>CI</th>
<th>USG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55.07±3.57</td>
<td>26.32±1.25</td>
<td>64.42±10.51</td>
<td>144.06±1.81</td>
<td>113.85±0.76</td>
<td>0.994±0.026</td>
</tr>
<tr>
<td>1</td>
<td>53.75±3.58</td>
<td>25.10±1.28</td>
<td>54.08±11.4</td>
<td>139.68±0.82</td>
<td>111.60±0.61</td>
<td>1.020±0.0015</td>
</tr>
<tr>
<td>3</td>
<td>49.61±3.62</td>
<td>24.02±1.46</td>
<td>46.64±10.20</td>
<td>141.84±1.09</td>
<td>113.77±0.88</td>
<td>1.022±0.0018</td>
</tr>
<tr>
<td>5</td>
<td>48.57±3.67</td>
<td>22.28±1.53</td>
<td>43.51±6.77</td>
<td>144.75±2.09</td>
<td>113.67±1.39</td>
<td>1.024±0.0013</td>
</tr>
<tr>
<td>8</td>
<td>43.3±5.22</td>
<td>21.3±1.92</td>
<td>38.01±7.46</td>
<td>144.57±1.98</td>
<td>144.73±0.92</td>
<td>1.024±0.0013*</td>
</tr>
<tr>
<td>11</td>
<td>28.72±3.85**</td>
<td>15.36±3.62**</td>
<td>45.12±11.24</td>
<td>167.98±1.35**</td>
<td>121.9±5.23</td>
<td>1.025±0.0012*</td>
</tr>
<tr>
<td>15</td>
<td>49.37±5.48</td>
<td>18.88±2.91**</td>
<td>53.31±17.34</td>
<td>143.72±4.04</td>
<td>117.26±3.07</td>
<td>1.023±0.001</td>
</tr>
<tr>
<td>20</td>
<td>48.55±6.55</td>
<td>20.80±5.70</td>
<td>145.30±69.20**</td>
<td>169.55±26.05**</td>
<td>126.65±7.05**</td>
<td>1.023±0.001</td>
</tr>
</tbody>
</table>

Units: Total Protein (TP)-g. L^{-1}; Albumin (ALB)- (g. L^{-1}); Uric Acid (UA)- mmol/L^{-1}; Sodium (Na)- mmol/L^{-1}; Chloride (Cl)- mmol/L^{-1}; Urine Specific Gravity (USG).

N.B. *Indicates that correlation is significant, P<0.05 level
** Indicates that correlation is highly significant, P<0.01 level.
**DISCUSSION**

This study was conducted with the goals to determine the degree of renal dysfunction, quantitatively and qualitatively, after experimental unilateral ureteral obstruction in dogs; to find a correlation between sonographic findings and laboratory indices (i.e., hematological, biochemical, urinalysis, and histopathological); to categorically grade the severity of ongoing destructive changes as per histopathological evidence, and subsequently to facilitate diagnosis and monitoring of affected patients.

Untreated or undiagnosed unilateral obstructive uropathy in pets can lead to a self-perpetuating permanent renal damage and chronic renal failure, by inciting hydronephrosis, which causes pressure atrophy of the kidney tissue (Kahn et al., 2005; Vegad and Katiyar, 1998). Derangements in the hematologic and biochemical indices are initially masked by compensatory responses; however, ultrasonography enables the clinician to visually detect changes in renal architecture, even with subtle renal derangements. Nevertheless, sonography does not quantify renal damage and quantitative assessments necessitate evaluation of severity of renal dysfunction.

Sonography revealed enormous changes in renal architecture qualitatively, thus confirming 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; D’Anjou et al., 2011; Carter et al., 1980). After two weeks of the experimental study, the size of the renal pelvis increased to about 3.54 times its normal size ($P<0.01$); 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 ($P<0.01$)- Chart 1. Most importantly, not only the sonographic changes correlated strikingly with changes in laboratory parameters, rather, through regression analysis, the degree of histopathological damage could be predicted from sonographically measured changes in renal parameters.

The hematological profile showed a normocytic, normochromic anemia with mild reticulocytosis, which has also been documented by other scientists and the etiology is attributed to the persistent, progressive, chronic inflammation and chronic renal insufficiency. On correlation analysis, parameters of RBC no., Hemoglobin (HgB) and hematocrit (HCT), showed a highly significant inverse correlation with renal length, width and depth, respectively (Table 2). Simultaneously, a low hematocrit (HCT) with lowered total protein (TPP) indicated substantial ongoing or recent blood loss (Meyer and Harvey, 2004), as during estimation of the kinetic parameters which were a part of the experimental study. Furthermore, significant leucocytosis and granulocytosis due to neutrophilia with a regenerative left shift, also proved indicators of the inflammatory responses immediately incited by the hydronephrotic kidney subsequent to the renal insult; whereas, moderate lymphocytosis in the initial stages, was attributed to increased lymphopoiesis subsequent to chronic antigenic or cytokine stimulation; additionally, a significantly severe thrombocytopenia observed on Day 11 was found to coincide well with the clinical evidence of a prolonged bleeding tendency after venipuncture (Stockham and Scott, 2008).

The biochemistry profile was suggestive of compensatory reparative mechanisms of the body, although an array of electrolyte and acid-base disturbances could be detected due to impaired excretory function. Marked hypoproteinemia and hypoalbuminemia were attributed to the poor body status, failure to synthesize more effective proteins, and blood loss. Hypoalbuminemia secondary to renal dysfunction on correlation analysis revealed a highly significant inverse correlation with renal length and depth (Table 2). Other indicators of renal dysfunction included creatinine and BUN, initial spikes of which indicated onset of renal dysfunction. However, all values remained within normal range, an evidence of an intact contralateral kidney and compensatory mechanisms of the body. Contrarily, Uric Acid proved a much promising indicator, the increase of which at a later stage of the experimental period ($P<0.01$

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Renal Length</th>
<th>Renal Width</th>
<th>Renal Depth</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Corpuscles (No.)</td>
<td>-0.8816**</td>
<td>-0.7846**</td>
<td>-0.8058**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (HgB)</td>
<td>-0.8824**</td>
<td>-0.7883**</td>
<td>-0.8125**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit (HCT)</td>
<td>-0.8598**</td>
<td>-0.79683**</td>
<td>-0.8199**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.8156**</td>
<td>-0.5348NS</td>
<td>-0.8355**</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.5047NS</td>
<td>0.3138NS</td>
<td>0.6436*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Urine Specific Gravity</td>
<td>0.852**</td>
<td>0.9068**</td>
<td>0.7763**</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Renal Histopathology</td>
<td>0.928**</td>
<td>0.699NS</td>
<td>0.952**</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Results of Correlation Analysis showing Strong Correlations of Sonographic Renal Parameters with Various Laboratory Parameters.
by Day 20) was suggestive of an established renal disease and metabolic acidosis in affected animals.

Kidney disease has also been postulated to predispose the animal to metabolic hyperchloremic acidosis, respiratory alkalosis and possible HCO3 deficits. Furthermore, changes in plasma chloride are generally paralleled by changes in plasma sodium. In agreement with the findings reported by Evans (2009), on Day 20, persistence of a highly significant serum chloride value was suggestive of hyperchloremia with possible HCO3 deficits and inadequate tubular functions, also indicating ineffective compensation by the body. This, combined with the sharp significant hypernatremia (P<0.01) evidenced on Days 11 and 20, was clearly indicative of the animals moving towards metabolic acidosis. This was further linked to a number of causes including low ECF volume (hypovolemia), renal tubular acidosis (Evans, 2009) and renal failure (acute and chronic) (Meyer et al., 1992). Hypernatremia secondary to the progressive renal dysfunction was finally evidenced through a strong correlation with (increase in) renal depth (Table 2).

Urinalysis examination revealed significant derangements in urine specific gravity on days 2, 6, 8, 10 and 12. These results indicated extensive cellular damage and subsequent lowered tubular concentrating ability. Like other parameters, changes in urine specific gravity showed strong correlation to ultrasonographic parameters of depth, length and width; thus, establishing diagnostic value in progressive renal disease.

Histopathological findings were strongly correlated to the sonographic increases in renal length and depth, respectively (P<0.001)- Table 2. Progressive renal atrophy through programmed cell death (apoptosis) was clearly visible in histopathologic sections of the affected tissues, as also reported by Tanji et al. (1998). Furthermore, the sections were characterized by severe tubular dilatation, significant tubular atrophy, hemorrhage and widened interstitial space with a greater number of interstitial cells and infiltrating mononuclear cells. Furthermore, tubulointerstitial fibrosis was the prominent feature of stained specimens in the later stages of the experimental study, as also documented by Wen et al. (1999). These changes were justified by the facts that obstructive uropathy not only causes pressure atrophy of the collecting system, but rather incites a sequence of complex pathophysiological changes such as infiltration of monocytes and macrophages in the renal cortex and medulla, activation of the rennin-angiotensin system, and alterations in the renal growth factor expression, ultimately culminating with a prominent change of permanent tubulointerstitial fibrosis (Ishidoya et al., 2003).

The most striking findings of this study were the prediction of grades of ongoing histopathological damage to the affected kidneys, through sonographic measurements of length and width, respectively. Regression formulae, derived for this purpose, can be used futuristically for prediction of severity of renal disease through simple non-invasive ultrasound measurements.

**Conclusion:** It can thus be concluded that prolonged unilateral urine outflow obstruction predisposes the kidneys to a complex cascade of structural and functional damage, however, the intact contralateral kidney compromises through compensatory responses which mask ongoing systemic derangements and cumulatively minimize the total loss in GFR. However, histopathologically, the renal tissue degenerates at a faster rate than is grossly visible through B-mode sonography. The severity of renal disease can now be predicted from simple non-invasive sonographic measurements. Despite that, timely implementation of latest diagnostic modalities can prevent aggravation of an otherwise reversible malady.

**Acknowledgments:** This work was part of a broader research project conducted at Nanjing Agricultural University, P. R. China; findings on prediction of changes in Glomerular Filtration Rate (GFR) through sonographic measurements using specific formulae have been published earlier (Bokhari et al., 2012). This paper correlates sonographic findings with laboratory data, specifically predicting ongoing degree of damage to affected kidneys through sonography. This work was successfully completed with award of a PhD degree by Nanjing Agricultural University (NJAU), to the author.

**REFERENCES**


