Possible role of calcium, phosphorous and magnesium shift in blood, urine and calculi in calves affected by urolithiasis

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Clinical study over a period of seven months at referral University Veterinary Hospital in Kashmir, India, was done on male calves clinically affected with urolithiasis was undertaken to manage them surgically and to study the mineral and haemato biochemical profile in an attempt to find out the probable cause of the malady and to ascertain the possible role of calcium, magnesium and phosphorous shift in urine blood and calculi. During obstructive urolithiasis, alterations in haematobiochemical profile were of no clinical significance, however, increased blood urea nitrogen (BUN), Creatinine, Calcium, and Inorganic Phosphorus could form a good index to determine the severity of the disease. Struvite calculi are most common urinary calculi observed in calves with heavy concentrate feeding especially wheat bran. The study revealed that the calculi contain 70% phosphorous, 20% calcium and 10% oxalate. This was clinically substantiated by the proportionate decrease in blood Ca and urine Ca levels. However, levels of creatinine and urea in urine blood and calculi revealed calcium phosphorous and their interrelation during the phase of urolithiasis, it could be conceived that ingestion of a ruminant feed containing high amounts of phosphates by a ruminant, physiologically with a non-functional rumen results in high absorption of the phosphates. This results in depletion of calcium absorption and calcium being excreted through urine.

Key words: Urolithiasis, Calves, biochemical, calculi.

INTRODUCTION

The urinary calculi turns clinically devastating when they obstruct the urinary conducts, result a disease condition known as obstructive urolithiasis. It may either be total or complete and partial, or incomplete with the resultant accumulation of urine and excretory metabolites in the urinary bladder. The retrograde pressure developed causes necrosis of the Urethral mucosa and leads to the perforation of the urethra, if the condition remains
Ruminants fed high-grain diets with low calcium to phosphorus ratio are at increased risk of developing struvite uroliths, low calcium: phosphorus ratio which augments urinary excretion of phosphorous and limited water intake or dehydration. While ruminants grazing on silica-rich soil are predisposed to form silica uroliths (Gianesella et al., 2010; Makhdoomi and Gazi, 2012).

The intracystic pressure develops in pace with duration of obstruction, depending upon the capacity of detrusor muscles the rupture of bladder occurs any time for 56 post obstructive hour onwards. In the event of rupture of the urethra, urine leakage occurs into the connective tissue of the ventral abdominal wall and prepuce resulting fluid swelling, cellulitis and urine scald exhibited by massive haematomas in ventral abdomen that can extend up to thorax. In event of rupture of the bladder, the patient experiences a slight relief from discomfort clinically but the excretory metabolites get accumulated in body and even increases so long as the cause is not corrected, the animal shows clinical signs of uraemia and toxæmia (Gazi et al., 2014).

If the condition is not diagnosed and corrected, uraemia continues to rise and the prognosis of the patient deteriorates and death becomes inevitable due to metabolic imbalances in which predominating conditions are hyponatraæmia, hypochloræmia, and possibly hyperkalaæmia (Radostitis et al., 2000).

MATERIALS AND METHODS

The study was conducted at Teaching and Veterinary Clinical Services Complex, SKAUST-K for a period of nine months in bovine calves aged 3 to 18 months manifesting the clinical urolithiasis. The mineral and haemato-biochemical profile of calves clinically affected with urolithiasis were taken into consideration and animals were managed surgically. An attempt has been made to find out the probable cause of the Urolithiasis and to ascertain the possible role of calcium, magnesium and phosphorous shift in urine, blood and calculi.

Grouping

On the basis of visible reflexes, plasma urea nitrogen and creatinine levels, ultrasonography of urinary system (Plate 1), duration of obstruction and position of calculi, the calves were divided into five groups of 6 animals each except one group having 3 animals only as under:

Group A (Alert clinically): They included animals with urinary obstruction of 24 h duration. They were clinically alert with viable reflexes, plasma urea nitrogen up to 50 mg/dl and creatinine 2-3 mg/dl respectively. They had intact bladder and the calculi were lodged in urinary bladder.

Group B (Below danger line): They included animals with urinary obstruction of 48 h duration, were clinically alert with sluggish reflexic, plasma urea nitrogen up to 50 to 100 mg/dl and serum creatinine 3 to 4 mg/dl respectively, with intact bladder (distended) and calculi were lodged in the neck of urinary bladder.

Group C (Critical): They included animals with urinary obstruction of 96 h duration. They were clinically dull with poor reflexic, plasma urea nitrogen above 100 mg/dl and serum creatinine 4 to 5 mg/dl respectively. They had ruptured bladder and the calculi were lodged in the neck of urinary bladder and sigmoid flexure (Plate 3).

Group D (Danger line): They included animals with urinary obstruction of 120 h duration. They were clinically recumbent with areflexic with plasma urea nitrogen above 150 mg/dl up to 200
mg/dl and serum creatinine 5 to 6 mg/dl respectively. The animals had ruptured bladder and calculi lodgement was in the neck of urinary bladder, ischial urethra and sigmoid flexure.

Group T (Terminal n=3): They included animals with urinary obstruction beyond 120 h duration. They were clinically recumbent, grossly areflexic with plasma urea nitrogen above 250 mg/dl and serum creatinine 7 mg/dl respectively. The animals had ruptured bladder and calculi lodgement was in the neck of urinary bladder, ischial urethra, sigmoid flexure and penial urethra.

Pre-operatively fluid and supportive therapy was given to animals with severe dehydration and or uremia as per the requirement of the case (Plate 4). The animals were allowed to stabilize and prepared for surgery at the earliest as per standard routine procedures suited to them as demanded by the clinical status of the animals. In all the groups during surgery, the calculi were removed and collected. They were dried in Whatman’s filter papers and the calculi were subjected to washing and drying. The calculus mass was weighed. The calculi were stored at 4°C in refrigerator till further analysis of their approximate composition. The chemical composition of the calculi was determined as per (Varley, 1988). The biochemical constituents of blood and urine viz, calcium, phosphorous, magnesium, urea nitrogen and creatinine also done on days 0, 8 and 16 day or on the day of removal of catheter. The urine samples were collected aseptically at the time of admission and 8th, 16th and at the time of removal of catheter post-operatively. The samples were filtered using Whatman’s filter paper no. 1 to remove any crystalline material and mucous etc. The samples were centrifuged and the supernatant taken for the estimation of Calcium (Kaser and Stekol, 1979), Phosphorus and Magnesium (Amador and Urban, 1977), urea nitrogen (Harold, 1976) and creatinine (Henry et al., 1974) using kits* while the sediment at the bottom was used for the microscopic examination of crystals. Samples were collected in heparinised vials and plasma was separated and subjected to Calcium (Kaser and Stekol, 1979), Phosphorus and Magnesium (Amador and Urban, 1977), Plasma urea nitrogen (Harold, 1976) and Creatinine (Henry et al., 1974) on days 8 and 16.

Statistical analysis

The data so procured was classified and subjected to statistical analysis. The inferences were drawn using analysis of variance (ANOVA) and Duncan’s Multiple Range test (Snedecor and Cochran, 1976).

RESULTS

Crystals

Heavy score of crystals was recorded in the animals of all groups on the day zero without any significant difference among them (Plate 2). Postoperatively the crystal showed progressive declining trend irrespective of the severity of the disease.

Biochemical results

The Mean±SE values of plasma urea nitrogen (mg/dl) and creatinine (mg/dl) in calves of different groups at different intervals are presented in Table 1. At day zero, plasma urea nitrogen was high (50.15±0.06 to 234.13±16.03) mg/dl. After instituting treatment, the levels of plasma urea nitrogen drastically decreased such that on day 8th, the average value of plasma urea nitrogen was 27.66±0.50 to 28.33±0.83 among different groups which varied insignificantly (P>0.05). At day 16th, the values ranged between 24.50±0.66 to 25.50±0.50 and at the time of removal of catheter, it ranged between 6.50±0.16 to 7.16±0.33. In general, there was significant (P>0.05) decreasing trend in the plasma urea nitrogen after the treatment irrespective of the groups. Higher than normal values for Plasma urea nitrogen were recorded during this study. These findings are in agreement with those of Villar et al. (2003); Tsuchiya and Sato (1991)

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The animals had ruptured bladder and the calculi were lodged in the neck of urinary bladder and sigmoid flexure.

Pre-operatively fluid and supportive therapy given to animals with severe dehydration and or uraemia.

and Gera and Nigam (1981) who also reported elevated levels of BUN values to the extent of 160 mg/dl (114.24 mmol/L), 120 mg/dl (85.00 mmol/L) and 181.96 mg/dl (129.91 mmol/L) respectively in cases of obstructive urolithiasis. The findings are also in agreement with those of Sheehan et al. (1994) who reported impaired
Table 1. Mean±SE of the plasma urea nitrogen (mg/dl) and creatinine (mg/dl) at different intervals in calves with obstructive Urolithiasis.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>8 day</th>
<th>16 day</th>
<th>*At the time of removal of catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alert clinically(A)</td>
<td>50.15±0.06aD</td>
<td>27.66±0.50aC</td>
<td>24.50±0.66aB</td>
<td>6.66±0.33aA</td>
</tr>
<tr>
<td>Below danger line(B)</td>
<td>86.55±4.93bC</td>
<td>28.00±0.33aB</td>
<td>24.83±0.50ab</td>
<td>6.66±0.33aA</td>
</tr>
<tr>
<td>Critical(C)</td>
<td>127.32±4.24cC</td>
<td>28.00±0.66ab</td>
<td>25.66±0.50ab</td>
<td>7.16±0.33aA</td>
</tr>
<tr>
<td>Danger line(D)</td>
<td>181.33±6.46dC</td>
<td>28.33±0.83ab</td>
<td>25.50±0.16ab</td>
<td>6.50±0.16A</td>
</tr>
<tr>
<td>Terminal(T)</td>
<td>234.93±16.03e</td>
<td>7.51±1.00e</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Above thirty days. Means with different superscripts differ significantly (P<0.01). Small letters show comparison between groups. Capital letters show comparison between treatments. Values in bold depict urea nitrogen levels.

DISCUSSION

In all the groups, the serum creatinine levels were more than the normal in all the groups with the severity of disease which varied significantly (P<0.05). Postoperatively, the creatinine values decreased progressively in all the groups from day eighth onwards, after removing urethral obstruction.

At day zero there was increasing trend in the serum creatinine with the severity of the disease and it ranged between 2.48±0.16 to 7.51±1.00 in clinically lesser to most severe cases. At day 8th, the average value of serum creatinine was 1.78±0.13 to 2.34±0.05 among different groups which varied non-significantly (P>0.05). At day 16th, the values ranged between1.56±0.10 to 1.39±0.13 and at the time of removal of catheter, it ranged between 1.13±0.09 to 1.10±0.03. In general, there was significant (P>0.05) decreasing trend in the serum creatinine after the treatment. The value for creatinine in the calves with obstructive Urolithiasis was much higher than the normal before treatment. This observation of the present study is in consonance with the findings of Gera and Nigam (1981) and Villar et al. (2003) who also reported higher values of creatinine in cases of obstructive Urolithiasis. Similar findings have also been reported by Singh et al. (1985) that in anorexia state (Watts and Campbell, 1971; Singh et al., 1985; Sockett et al., 1986). In the present study, the plasma levels of creatinine were higher in the cases of ruptured urinary bladder than in the cases of intact urinary bladder. These findings are in agreement with those of Smith (2002) and Donecker and Bellamy (1982) who reported more profound serum biochemical alterations in ruptured bladder cases than those without ruptured bladder. Higher values of creatinine in the ruptured cases could be due to movement of creatinine from the peritoneal fluid to blood (Donecker and Bellamy, 1982). Kerr (2002) reported that urinary creatinine levels clearance of urea and creatinine in urinary tract obstruction. Urea is a nitrogenous waste product, which is formed in the liver as the end product of amino acid breakdown and is excreted by kidney into urine (Kerr, 2002). In obstructive Urolithiasis, urine gets accumulated into the urinary bladder for more than normal period of time. The urea gets reabsorbed into the systemic circulation and causes uraemia. Blood urea nitrogen in the cases of ruptured urinary bladder was higher than that in the intact urinary bladder cases, which could be due to movement of urea from the high concentration in peritoneal cavity to the interstitial and intravascular compartments. The higher values for Plasma urea nitrogen in ruptured bladder cases are in total consonance with those of Smith (2002) and Donecker and Bellamy (1982) who reported more profound biochemical alterations in cases with ruptured bladder. In ruminants, large amount of urea is excreted in the saliva and enters into the rumen where it is hydrolysed by urease to ammonia, which is drained to venous blood but reconverted into urea by liver (Watts and Campbell, 1971). This mechanism may help in maintaining the blood urea level in normal range for some time but in long standing cases, high blood urea nitrogen may occur in spite of recycling of urea through rumen (Sharma et al., 2006).
Table 2. Possible role of calcium in urine, blood and calculi at different intervals of study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Onset phase</th>
<th>Middle phase</th>
<th>Recovery phase</th>
<th>Onset phase</th>
<th>Middle phase</th>
<th>Recovery phase</th>
<th>Proximate composition of calculi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine (mg/dl)</td>
<td></td>
<td></td>
<td>Blood (m mol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.83±0.06&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>9.36±0.20&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>11.42±0.46&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>1.36±0.05&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.41±0.09&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.05±0.01&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>Total ash 78.80%</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash 30.38%</td>
<td>Calcium 3.74%</td>
<td>Phosphorous 1.15%</td>
<td>Oxalate 1.35%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4.83±0.16&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>9.81±0.20&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>11.86±0.09&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>1.38±0.06&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.37±0.09&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.07±0.02&lt;sup&gt;AC&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4.96±0.23&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>9.83±0.14&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>12.02±0.09&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>1.38±0.06&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.37±0.06&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.07±0.02&lt;sup&gt;AC&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3.96±0.13&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>9.83±0.45&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>11.87±0.02&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>1.36±0.05&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.41±0.07&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.05±0.01&lt;sup&gt;AC&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means with different superscripts differ significantly (P<0.01). Small letters show comparison between groups. Capital letters show comparison between treatments.

elevate more quickly than urea levels at the start of the disease, and also decrease more quickly when an improvement takes place, thus it could be used for early diagnosis and recovery from the disease. However, during this study changes in BUN and urinary creatinine levels were almost similar and both could be used to ascertain the efficacy of treatment.

The mean ± SE values of plasma calcium (mmol/l) in the calves with obstructive Urolithiasis in different groups are represented in Table 2. Plasma calcium levels at 0 day were (1.30±0.05 to 1.38±0.06) lower than normal in all the groups. By day eighth, there was a progressive recovery toward normal in the plasma calcium levels and by day 16th irrespective of the severity of disease. The severity of the disease affect non- significantly (P>0.05) on the levels of plasma calcium at any stage of the disease. There was significant (P<0.05) increase in the urinary calcium levels from the day of admission of the calves in the clinics to the phase of recovery. The proximate composition of the calculi showed that they contained calcium 3.74% and the animals of groups D and T showed calcium/Amorphous phosphate, Calcium carbonate, Calcium oxalate, Uric acid 25 to 35%.

The mean±SE values of plasma phosphorous (mmol/l) levels in calves with obstructive Urolithiasis are presented in Table 3. At day zero hyperphosphataemia was recorded in all the groups. After instituting treatment, the plasma phosphorous followed a decreasing trend towards normal at days 8<sup>th</sup> and 16<sup>th</sup>. At the time of removal of catheter, the decrease was however, significant (P<0.05) decreasing trend in the inorganic phosphorous levels within normal range at day sixteenth irrespective of the severity of the disease. The study revealed that the levels of Phosphorous in urine and blood followed a similar trend. Increased levels of Phosphorous in all the groups ranged from 6.81±0.08 to7.15±0.06 in urine in all the groups, A to D at the onset phase, while it remained within 5.30±0.08 to 5.58±0.07 m mol/l in blood at the onset phase and showed a slight decrease afterwards. However, the decrease was
clinically within near normal range in all the four groups (2.57±0.103 group B and C and 2.65±0.03 group A and D) in blood. At the recovery phase, the levels of urinary Phosphorous reached to 5.83±0.21 in group A and 5.78+0.05, 6.01+0.08 and 6.21+0.16 in groups D, B and C respectively. An analysis of calculi revealed that the calcium levels were 3.74%.

Percentage of different crystals in urine in calves showed Prismatic, coffin lid shaped, star shaped elongated rod, feathery or fern like Triple phosphate crystals in A, B and C up to 65 to 75. While the proximate composition of the calculi Phosphorous was 1.15%.

The mean±SE values of plasma magnesium (mmol/l) levels in calves with obstructive Urolithiasis are presented in Table 3. At day zero increased values were recorded in all the groups. After instituting treatment, the plasma magnesium followed a decreasing trend towards normal at days 8th and 16th. At the time of removal of catheter, the decrease was however significant (P<0.05) decreasing trend in the plasma magnesium levels within normal range at day sixteenth irrespective of the severity of the disease. At day zero, there was increase in urinary magnesium levels with the severity of disease in all the groups under study.

However, at day 8th and at the time of removal of catheter, the variability levels in urinary magnesium among different groups was varying non-significantly (P>0.05). With the advancement of post-treatment period, there was significant (P<0.05) decreasing trend in the urinary magnesium levels within normal range at day sixteenth irrespective of the severity of the disease (Table 3). Inter relation of the magnesium in urine plasma and calculi goes, the studies revealed about (65 to 75%) of the calculi composed of magnesium ammonium phosphate and rest were comprised of calcium phosphate, calcium carbonate, calcium oxalate and uric acid.

**Possible role of calcium and phosphorous in urine, blood and calculi**

As far the role of calcium phosphorous and their interrelation during the phase of urolithiasis goes, it could be conceived that ingestion of a ruminant feed containing high amounts of phosphates by a ruminant, physiologically with a non-functional rumen results in high absorption of the phosphates which is clinically substantiated in Tables 2 and 3. This results in depletion of calcium absorption and calcium being excreted through urine. This is obvious from our previous findings of screening; wherein crystals of calcium have been found present well before the manifestation of clinical symptoms of urolithiasis. In absence of the acid base studies regarding the subject how the crystallization of the calculi takes place cannot be elucidated. Various factors come to play the role in the composition of the calculi, particularly geography, species, age, sex, composition of feed, pH of urine, urinary tract infection etc (Radostitis et al., 2000). During this study, composition of feed seemed to be the profound predisposing factor, as wheat bran alone or in combination with other feeding stuffs was given to the maximum number of calves. Rations high in grains but with limited amount of roughages leads to ammonium phosphate urolithiasis in feedlot cattle (Belknap and Pugh, 2002).

The feeding schedule of the calves revealed that they were fed concentrate mixture which was high in the phosphorous and low in calcium. The findings are in total agreement with those of Munakata et al.(1974a,b); Ahmed et al. (1989); Finlayson (1974) who reported that highly digestible, low roughage ration having more phosphorous than calcium e.g. high grain feeding lead to the formation of insoluble struvite calculi. Presence of calcium phosphate deposits between the struvite crystals represented the epitaxial growth, which signifies the growth of one type of crystal upon another type. Determination of chemical composition of the calculi revealed that they contain 70% P, 20% Ca and 10% oxalate. This was clinically substantiated by the proportionate decrease in blood Ca and urine Ca levels. Obstructive urolithiasis causes severe alterations in haematobiochemical profile that could be of no clinical significance, however, increased BUN, Creatinine, Calcium, and Inorganic Phosphorus could form a good index to determine the severity of the disease.

**Conflict of Interest**

The authors have not declared any conflict of interest.

**REFERENCES**