Biochemical evaluation of urine and serum sample of patients with nephrolithiasis in Pondicherry

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ABSTRACT

The urinary system is one of the excretory systems of the body. Nephron is the structural and functional unit of kidney. Kidney stones are one of the most painful of the urologic disorders and are one of the most common disorders of the urinary tract. The 5% of the population will have a clinical stone event at some time during their lives. A Kidney stone is a hard mass developed from crystals that separate from the urine and buildup on the inner surfaces of the kidney. In the present study, organic and mineral analysis was carried out in serum. Urine also analyzed for the quantification of oxalate, citrate, calcium and citrate. Sodium and phosphorous found to be decreased in Nephrolithiasis patients when compared to normal subjects. Calcium, potassium and citrate found to be elevated. Uric acid is not significantly changed in serum when compared with normal. In urine, oxalate and calcium excretion increased whereas citrate excretion decreased. In the case of uric acid no significant change occurs in excretion amount. From the results, it was concluded that the patients were affected by calcium and oxalate stones.

INTRODUCTION

The urinary system is one of the excretory systems of the body. They are two kidneys which secrete urine, two ureters which convey the urine from the kidney to the urinary bladder, one urinary bladder where urine collects and is temporarily stored and one urethra through which the urine is discharged from the urinary bladder to the exterior. Nephron is the structural and functional unit of kidney (Guyton, 2000).

Kidney stones

Kidney stones are one of the most painful of the urologic disorders and are one of the most common disorders of the urinary tract. In 2000, patients made 2.7 million visit to health care providers and more than 6,00,000 patients went to emergency rooms for kidney stone problems. Men tend to be affected more frequently than women. In industrialized, relatively affluent populations, renal stone formation (nephrolithiasis) has increased in frequency, while bladder stone formation has almost disappeared as the prevalence of malnutrition and infection have decreased. The 5% of the population will have a clinical stone event at some time during their lives. Stone formation occurs in men about four times as frequency as in women and in both sexes tends to be both recurrent and unpredictable. Investigation and treatment of stone formers is undertaken to prevent recurrent stone formation, but in general permanent intrinsic renal damage does not occur unless there is superadded infection in an obstructed kidney (Tietz, 1995). Urolithiasis (stones in the urinary tract) is a common medical problem with a prevalence of around 2–3% in the general population (Srisubat et al., 2009).

A Kidney stone is a hard mass developed from crystals that separate from the urine and buildup on the inner surfaces of the kidney. Normally urine contains chemicals that prevent or inhibit the crystals from forming. These inhibitors do not seen to work for everyone however, so some people form stones. If the crystals remain tiny enough, they will travel through the urinary tract and pass out of body in the urine without being noticed. The most common type of stone contains calcium in combination with either oxalate or phosphate (45%). A less common type of
Stone is caused by infection in the urinary tract. This type of stone is called a struvite or infection stone. Cystine stones are rare (William Marshall, 1989).

Structure of Renal Calculi

Renal calculi may be as small as a grain of sand or as large as a pearl. Some stones are even as big as golf balls; stones may be smooth or jagged. They are usually yellow or brown.

Types of Renal Stones

A number of factors can cause changes in your urine, including the effect of local climate, diet, occupation, stress and some metabolic and genetic disorders may influence the likelihood of stone formation. There are different types of kidney stones include cystine stones, oxalate stones, uric acid stones, struvite stones and phosphate stones (Wasserstein, 2005).

<table>
<thead>
<tr>
<th>Type of Stones</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium oxalate and phosphate</td>
<td>45%</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>35%</td>
</tr>
<tr>
<td>Uric acid</td>
<td>5%</td>
</tr>
<tr>
<td>Cystine</td>
<td>1 - 3%</td>
</tr>
<tr>
<td>Cap</td>
<td>1 - 2%</td>
</tr>
</tbody>
</table>

Analysis

Both qualitative and quantitative analysis of the chemical consistent of kidney stones may be useful in establishing their origin and in planning relational therapy. Analysis is not needed for every urinary calculus when other laboratory and clinical finding are diagnostic. In some patient’s however, two or more possible etiologies exist, and analysis of the calculus may help distinguish between different diagnosis. Oxalate stones may develop renal infections with deposition of magnesium ammonium phosphate upon the calcium oxalate stones to form "mixed stones". Chemical analysis of these stones is needed to conform this sequence of events. Specialized physical techniques such as infrared spectroscopy and X-ray diffraction are gradually replacing less specific, qualitative chemical methods for stone analysis.

Nephrolithiasis is a common disorder that accounts for significant cost, morbidity, and loss of work. In the United States, the estimated annual expenditure for the diagnosis and treatment of nephrolithiasis approached $2.1 billion in 2000, almost certainly an underestimate, without taking into account lost wages and reduced work productivity. Indeed, there is a one in eight lifetime chance of being diagnosed with urinary stones. Although in most cases stones are source of discomfort and inconvenience without significant risk to health, progressive loss of renal function can occur after repeated episodes of stone disease. In a French study of over 1300 patients newly requiring hemodialysis, 3.2% of cases were directly related to stone disease. With comprehensive evaluation, metabolic abnormalities can be identified in over 90% of stone formers, and the institution of preventive dietary and medical measures has resulted in substantial reduction in stone recurrence rates. A careful medical and dietary history, serologic tests, and urinalysis constitute the initial screening tools in stone formers.

Hence, in the presence study an attempt has been made to identify the biochemical parameters changes in nephrolithiatic patients. For this purpose, biochemical parameters were planned to be analysed in both blood and urine sample. In this context, organic parameters like citrate, oxalate and uric acid were selected and mineral parameters like sodium, potassium, calcium and phosphorous were selected. Citrate, uric acid, sodium, potassium, calcium and phosphorous were planned to analyze in serum whereas calcium, oxalate, uric acid and citrate were planned to analyse in urine sample.

Materials and Methods

Sample was collected from the Normal individuals and Patients with Nephrolithiasis from Puducherry. They were same sex and age between 30-40 years. From them 10-15ml of blood sample were collected. Urine sample was also collected for 24hrs and used for analysis. Blood samples collected from Normal and Patients were allowed to clot at room temperature for 15 minutes, the serum was separated and poured into small sterile eppendorff tubes. The Serum was stored in the refrigerator at 4°C till use. Urine sample was preserved by adding 10ml of Hydrochloric acid.

Citric acid estimation was done using the colorimetric method based on oxidation of citric acid in urine to

Figure 2.1 Kidney stones (Courtesy to: http://www.aasthahealthcare.com/images/stones-in-ureter.jpg)
Serum Analysis

Table 1: Result of Organic Analysis of Controls and Nephrolithiasis Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Organic Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citrate* (mMol/L)</td>
</tr>
<tr>
<td>Study Group</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0.08 ± 0.0</td>
</tr>
<tr>
<td>Group II</td>
<td>0.10 ± 0.0</td>
</tr>
</tbody>
</table>

Table 2: Result of Macrominerals Analysis of Controls and Nephrolithiasis Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Macrominerals Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium (Meq/L)**</td>
</tr>
<tr>
<td></td>
<td>Potassium (Meq/L)*</td>
</tr>
<tr>
<td></td>
<td>Calcium (mg%)**</td>
</tr>
<tr>
<td></td>
<td>Phosphorous (mg%)**</td>
</tr>
<tr>
<td>Study Group</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>139.60 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>3.29 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>8.98 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>3.66 ± 0.32</td>
</tr>
<tr>
<td>Group II</td>
<td>131.0 ± 3.60</td>
</tr>
<tr>
<td></td>
<td>5.06 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>11.70 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>2.74 ± 0.20</td>
</tr>
</tbody>
</table>

Urine Analysis

Table 3: Result of Urine Analysis of Controls and Nephrolithiasis Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urine Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxalate mMol/d**</td>
</tr>
<tr>
<td></td>
<td>Citrate mMol/d**</td>
</tr>
<tr>
<td></td>
<td>Calcium Mg/d**</td>
</tr>
<tr>
<td></td>
<td>Uric Acid Mg/d</td>
</tr>
<tr>
<td>Study Group</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0.55 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>2.00 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>134.60 ± 10.90</td>
</tr>
<tr>
<td></td>
<td>311.20 ± 44.10</td>
</tr>
<tr>
<td>Group II</td>
<td>1.26 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>232.00 ± 19.20</td>
</tr>
<tr>
<td></td>
<td>325.00 ± 42.00</td>
</tr>
</tbody>
</table>

Group I - Controls and Group II - Nephrolithiasis patients.
Data were expressed as Mean ± SD.
*P < 0.05 statistically significant level.
**P < 0.001 statistically significant level.
Statistical Analysis

Statistical analysis was performed on a personal computer using the SPSS software evaluation version 15.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD). Data were analyzed using independent t test by applying mean comparison method (Blalock, 1972). Significance difference were defined as two tailed P<0.05 and P<0.001.

Results and Discussion

Renal calculi or renal stone disease covers many conditions causing kidney, ureteric or bladed stones. These include metabolic and inherited disorders, anatomiced defects of the upper or lower urinary tract, and chronic urinary infection. Most cases of renal stones are idiopathic and present in loin or abdominal pain.

Table-1 shows the parameters of citrate and uricacid levels in serum of normal and patients. The serum level of citrate was 0.08 ± 0.0 and 0.10 ± 0.0 respectively. Even though, there was a slight elevation present in patients, the values still present well within the normal values. There was only P<0.05 level of significance. There was no much experimental evidence available for the increase in serum citrate level in patients.

Table-2 showed the levels of sodium, potassium, calcium and phosphorous in renal patients and in normal subjects. Level of sodium in normal and nephrolithiasis patients was represented in the Table-2. Values of sodium in normal and patients were 139.60 ± 2.30 and 131.0 ± 3.60 respectively.

The present study results have correlated with that reported by Madore. Higher dietary intake of the sodium has been associated with the higher risk of hypertension and also may increase the risk of nephrolithiasis (Madore et al., 1998). Potassium in the presence of adequate sodium consumption results in sodium rention and hypercalcuria (Shey et al., 2004). The study results provided the following values for normal and patients 8.98 ± 0.34 and 11.70 ± 0.66 respectively. There was a marked increase present in serum calcium levels. The statistical level of significance found to be P<0.001. Present study results have correlated with that reported by Heller, who also reported that there is an increased level of calcium which leads to hypercalcuria (Heller et al., 1999).

The phosphorous levels of present study were 3.66 ± 0.32 and 2.74 ± 0.20 respectively. The phosphorous level was slightly decreased than normal persons. The effect might be due to the effect of its counter ion calcium in circulation. The level of phosphate in serum is decreased in renal calculi patients when compared with normal subject due to the decrease in the capacity of kidney to reabsorb phosphate. It leads to Hypophosphaturia and osteomalacia. Hypokalemia may reduce phosphate reabsorption by the renal tubule causing hypophosphatemia (Audran and Legrand, 2000). Hyperphosphaturia in patients with urolithiasis has been reported by several authors. However, the frequency of a low phosphorous with normal parathyroid function was unknown (Prie et al., 2001). Phosphate deficiency is associated with increased production by the kidney of the active form of vitamin D which increases intestinal absorption of calcium which indirectly increases excretion of phosphorous and decreasing phosphorous level in serum (Audran and Legrand, 2000).

Table-3 showed the urinary levels of oxalate, citrate, calcium and uric acid in renal patients and in normal subjects. The present provided the following level of oxalate values for normal and patients 0.55 ± 0.05 and 1.26 ± 0.10 respectively. Oxalate excretion found to be almost doubled than normal persons. The statistical significance level was P<0.001. Present study results have correlated with that reported by Massey who also reported that there is an increased level of oxalate which leads to hyperoxaluria. Ascorbate increases human oxaluria and kidney stone risk Supplementation may increase the risk of kidney stones (Massey et al., 2005). Primary hyperoxaluria is a rare autosomal recessive disorder that may be present in adult life. The disease should be in the differential for any patients who presents with renal failure and live directerioris (Bogle et al., 2003). A possible candidate for the gene influencing oxalate excretion is one associated with the increase in oxalate self exchange noted in erythrocytes isolated from a large percentage of individuals with calcium oxalate nephrolithiasis (Goodman et al., 1997).

The levels of citrate in normal subjects and in renal calculi patients in urine values were 2.00 ± 0.21 and 0.82 ± 0.08 respectively. The values were decreased in patients with renal calculi when compared with normal subjects. The decrease was because of the increase in calcium level in urine. The present study
results have correlated with that reported by Cupisti and it was reported that there is a decreased level of citrate excretion in calcium stone formers. Due to the insulin resistance may contribute, be lowering urinary citrate excretion and thus to an increased risk of stone formation (Cupisti et al., 2007). Hypocitraturia was found as a common risk factor associated with recurrent calcium stone formation and associated with low urinary potassium level, low alkaline absorption and high titrable acid excretion. Hypocitraturia is predominantly of dietary origin (Domrongkitchaiporn et al., 2006). Citrate has been widely studied for its stone inhibiting action in urine and it has been found to be particularly effective against the calcium oxalate and phosphate stone. An incidence of hypocitraturia in 46% of stone formers is reported (Ratan et al., 2002). The clinical effects of the citrate contents of citrus fruits such as lemon and orange on the hypocitraturia in stone patients were studied and it was found that they have positive effects on kidney stone formation (Yilmaz et al., 2006). Low levels of urinary citrate, an important inhibitor of calcium stone formation, may contribute to the increased risk for kidney stone disease (Taylor and Curhan, 2006). Hypokalemia, drugs like thiazide, Azetazolamide and Acidosis decrease the urinary citrate excretion (Hamm and Hering-Smith, 2002).

Urinary calcium excretion in normal and nephrolithiasis patients were 134.60 ± 10.90 and 232.00 ± 19.20 for normal and patients respectively. The level of calcium is increased in renal calculi patients when compared with normal subjects. The level of statistical significance was found to be P<0.001. Hypercalciuria is a risk factor for kidney stone formation and low bone mass. The dietary calcium increases the urinary calcium. Bone loss seen in hypercalciuric patients. A diet high in animal protein stimulates bone resorption and increased sensitivity to this and other dietary factors may cause hypercalciuric patients to lose bone. High calcitriol levels in a group of patients with hypercalciuria and osteopenia (Audran and Legrand, 2000). Hypercalciuria is classified according to the site of the primary defect in calcium transport; secondary changes can occur at other sides, which are renal calcium lack leads to secondary hyperparathyroidism, which results in bone resorption and increased intestinal calcium absorption (Park and Pearte, 2005). The Topiramine treatment increases the urinary calcium excretion. Increase the propensity to from calcium stones (Welch et al., 2006). The diet characterized by normal calcium, low animal protein and low salt levels is more effective that the traditional low–calcium diet for the prevention of recurrent stones in men with idiopathic hypercalciuria (Borghi et al., 2002).

The Urinary excretion of uric acid in normal and nephrolithiasis study groups were 311.20 ± 44.10 and 232.00 ± 42.00 respectively for normal and patients. But the increase in nephrolithiasis patients was not statistically significant. The present study results have correlated with that reported by Pak and it was reported that there is an increased level of calcium leads to Hyperuricosuricosa. Stone-forming patients with idiopathic uric acid nephrolithiasis were shown to have biochemical features of primary Gout, characterized by low urinary PH and fractional excretion of urate (Pak et al., 2001). Hypercuricosuria also occurs in rare, hereditary enzymatic disorders including hypoxanthine guanine phosphoribosyl transferase (HPRT) deficiency Glycogen storage diseases, muscle phospho fructo deficiency (Cameron and Sakhaee, 2007). Gibney has reported the relative contributions of these mechanisms to increase in urinary uric acid excretion remain unclear, and the importance of hypercuricosuria or uric acid supersaturation to the increased incidence of bone disease is uncertain (Gibney and David, 2003). Matrix has reported the prevalence of gout was greater in individuals with previous kidney stones (Kramer and Curhan, 2002). Eventhough several reports suggested the elevation in the excretion in different calcium stone patients, in the present study the uric acid excretion was not increased significantly.

Conclusion

In the present study, organic and mineral analysis was carried out in serum. Urine also analyzed for the quantification of oxalate, citrate, calcium and citrate. Sodium and phosphorous found to be decreased in Nephrolithiasis patients when compared to normal subjects. Calcium, potassium and citrate found to be elevated. Uricacid is not significantly changed in serum when compared with normal. In urine, oxalate and calcium excretion increased whereas citrate excretion decreased. In the case of uric acid no significant change occurs in excretion amount. From the results, it was concluded that the patients were affected by calcium and oxalate stones. They may not be affected by uricacid stones because of the absence of significant change in the uric acid level in both serum and urine. Decreased citrate levels in urine excretion also an indication for calcium stones. These patients were suffering from hypercalcemic, hyperoxaluric and hypocitraturic condition. Further investigations required to find out exact reason for the calcium stone formation and proper treatment. Extension of this study might provide a new face for pathophysiology of Nephrolithiasis patients and it also helpful to reduce major complications of Nephrolithiasis patients with special reference to electrolytes and organic substances like oxalate, urate and citrate.

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