ANALYSIS OF 24 HOURS URINARY CITRATE, CALCIUM, PHOSPHOROUS, MAGNESIUM & URIC ACID LEVELS IN PATIENTS WITH UROLITHIASIS

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ABSTRACT

Background: Urolithiasis denotes stones originating anywhere in the urinary tract, including the kidneys and bladder and is a worldwide problem sparing no geographical cultural, or racial groups. Urinary stones usually arise because of the breakdown of a delicate balance between solubility and precipitation of salts.

Aim & Objectives: To analyse 24 hrs urinary citrate, calcium, phosphorous, magnesium & uric acid levels in patients with Urolithiasis.

Methodology: The study consists of fifty patients with evidence of renal stone disease and fifty age and sex matched healthy controls. Subjects for the study were selected from outpatients attending Vinayaka Missions Kirupananda Vairayar Medical College & hospital, Salem., Tamil Nadu. Twenty-four hours urine samples were collected for the analysis of Volume, pH, urinary citrate, calcium, phosphorous, magnesium & uric acid levels.

Results: 24 hours urine volume in cases were significantly lowered when compared to control group. Urine pH in cases (<5.5) was significantly lowered when compared to control group (>6). 24 hours Urinary citrate, phosphorous and Magnesium levels were significantly lowered in cases when compared to control groups. Citrate 271.78 ± 26.72 mg/day Vs 739 ± 228.12 mg/day, Magnesium 39.227 ± 4.287 mg/day Vs 116.6 ± 45.74 mg/day. Phosphorous 251.12 ± 29.4 Vs 688.84 ± 162.6 mg/day in cases and controls respectively. 24 hours urinary calcium in control group 170.92 ± 35.32 and in cases 291.76 ± 36.03. Calcium in cases were significantly higher when compared to control. (p value <0.001). 24 hours urinary uric acid in control group 501.68 ±136.79 and in cases 819.66 ± 40.307. Uric acid in cases were significantly higher when compared to control. (p value <0.001).

Conclusion: There was a significant decrease in urinary citrate level in renal stone formers compared to healthy controls, suggesting that hypocitraturia is an important metabolic risk factor for renal stone formation.

Key words: Urinary citrate, calcium, phosphorous, magnesium, uric acid, Urolithiasis.

INTRODUCTION

Renal Calculus Disease is a common disease that has been recognized and documented in medical literature since the Greek and Roman physicians. It is a multifactorial disease caused by the alteration of normal crystallization conditions of urine in the urinary tract. It is a recurrent disease of worldwide distribution and occurs in rural, urban, industrial and non-industrial regions. The epidemiology of
Urolithiasis differs according to geographical and climatic aspects in terms of prevalence and incidence, age and sex distribution, occupation, diet, hereditary and metabolic factors, stone composition and stone location. Renal pelvic stone was more common in the female (22%) than in male (8.5%) patients. Spontaneous passage of stone occurred in 87.5% of the men and in 81.5% of the women.

There was a relationship between stone disease and gout and also relationship with positive family history. Stone formers used less water from public aqueducts and more uncarbonated mineral water. The rate of renal stone diseases occur in males is slightly higher than females, and in white people than Blacks. A high frequency of stone formation among hypertensive patients has been reported, and among those with high body mass as well.

The risk factors for Urolithiasis include an individual's susceptibility to form stones, such as genetic predisposition and metabolic abnormalities, and environmental factors that facilitate stone disease, such as dietary practices as well as local climate characteristics. Prevalence of Urolithiasis varies in different countries. In adults the prevalence is relatively higher in Western countries than in the Eastern hemisphere.

Metabolic factors and family history, environmental factors are more common in paediatric than adult stone formers. Countries in the Afro-Asian stone belt falling within the tropical and subtropical regions have consistently reported a high incidence of urolithiasis. Several studies indicate that urinary stone disease has a high prevalence in India. In India approximately 5-7 million patients suffer from stone disease and at least 1/1000 of Indian population needs hospitalization due to kidney stone disease. A recent survey shows that there has been rapid increase in the number of people suffering from Kidney stones.

Renal stones form twice as often in men as women. The prevalence of kidney stones begins to rise when men reach their 30s, and it continues to increase with age, but women have a bimodal age distribution, with peaks at 35 and 55 years. Once a kidney stone forms, there is a 50% probability that a second stone will form within five to seven years. A seasonal variation is also seen, with high urinary calcium oxalate saturation in men during summer and in women during early winter. Stones form twice as often in men as women. The western region of Saudi Arabia is an area with a high prevalence of Urolithiasis. This study was designed to find the effect of climatic changes on the occurrence of urinary stone formation as well as the effect of Ramadan fasting and pilgrimage festival. Increase in renal stone colic in hot summer season than winter season. A strong correlation was found between urinary stone colic and both temperature and atmospheric pressure.

Variety of factors are associated stone formation including gender, diet and urinary excretion of calcium, uric acid and oxalate. Several of these factors may be related to body size. Height was inversely associated with the prevalence of stone disease but was not associated with incident stone formation. These results suggest that body size is associated with the risk of stone formation and that the magnitude of risk varies by gender.

The risk of stone disease is known to be correlated with various environmental factors such as climate, socioeconomic status, geography, dietary habits and obesity. Numerous reports have also noted genetic correlations such as sex, age, race, idiopathic hypercalciuria, hyperoxaluria, and hyperuricosuria. Also about 25% of patients with Urolithiasis have a family history of stone disease. The main difference between stone formers and healthy subjects were that stone formers had a family history of stones, a higher body weight, a lower daily intake water, calcium, and a higher urinary output of calcium and oxalate. These results represents the combined role of genetic and nutritional factors in the pathogenesis of urinary stone formation.
A smaller urine volume does increase urine supersaturation with regard to the calcium-containing solid phases, which constitute the majority of kidney stones. Diet rich in spinach, tomatoes, large quantity of groundnuts, roti, and frequent non-vegetarian diet, bore-well (hard) water are more prone to cause renal calculus Disease. However, a family history of kidney stones (increases risk by three times), insulin resistant states, a history of hypertension, primary hyperparathyroidism, gout, chronic metabolic acidosis, and surgical menopause are all associated with increased risk of kidney stones. Incidence of stones is higher in patients with an anatomical abnormality of the urinary tract that may result in urinary stasis. Though there are risk factors for causation of renal stone disease, there are inhibitory factors preventing Urolithiasis. The well-known urinary inhibitors are citrates, magnesium, pyrophosphate, Tamm-Horsfall proteins, glycosaminoglycans, osteopontin (uroptin) and high urine flow. While renal stone promoters are calcium, sodium, oxalates, urates, cystine, low urinary pH and low urine output.

Low urinary citrate excretion is a known risk factor for the development of kidney stones. Citrate inhibits stone formation by complexing with calcium in the urine, inhibiting spontaneous nucleation, and preventing growth and agglomeration of crystals. Hypocitraturia is a common, metabolic abnormality found in 20% to 60% of stone formers. It is now frequently recognized that hypocitraturia is an important and correctable biochemical abnormality present in 15% to 63% of patients with renal stone disease. Urinary citrate levels are generally higher in women than in men. In hypocitraturia, citrate excretion is less than 100 mg for men and 200 mg for women. Hypocitraturia enhances urine calcium salt supersaturation and reduces calcium crystallization inhibition, increasing the risk of calcium nephrolithiasis. It also may play a role in uric acid solubility and uric acid stone formation.

The purpose of the present study is to compare the 24 hour urinary citrate levels in renal stone formers and the normal individuals in this area and to define the role of urinary citrate levels in the etiology of renal stone formation.

**MATERIALS AND METHODOLOGY**

The study consisted of fifty patients with ultrasound evidence of renal stone disease and fifty age and sex matched healthy controls. Subjects for the study were selected from outpatients attending Vinayaka Missions Kirupananda Variyar Medical College & hospital, Salem., Tamil Nadu Institutional ethical committee clearance and consent from the patients were obtained.

The Control Group included Fifty healthy individuals and 50 urolithiasis subjects with age group ranging between 25-50 years of both sexes were selected for the study. Patients with history of Hypertension, Diabetes, Congestive heart failure, Gouty arthritis, HIV and fever were excluded from this study.

The patients with Urolithiasis for the first time were included in this study and the diagnosis was made by Ultrasonography.

Twenty-four hours urine samples were collected in a wide capped clean transparent graduated plastic collection bottle. The preservatives used are 10N Hydrochloric acid was used for calcium, 10N sulphuric acid was used for citrate and Thymol crystals was used for sodium, uric acid, magnesium and phosphorus.

The samples were preserved in refrigerator and analysed. Morning voided urine was collected and pH was determined by using pH meter and 24 hours urine volume was measured. Calcium, Citrate, Uric acid, Magnesium and Phosphorous were estimated in 24 hours urine samples of control and cases.
Citrate levels were estimated in urine by quantitative method using MPA (Meta Phosphoric Acid), citrate reagent and petroleum ether by using acid reagent (Esteem kit). Final absorbance was taken in [micro lab 300] Semiauto analyser at 405 nm. Magnesium levels were estimated in urine by xylyl blue method, Phosphorous levels were estimated in urine by [Randox] end point method.

Estimation of uric acid in urine done by enzymatic photometric method using TBHBA (2,4,6-tribromo 3 hydroxy benzoic acid) and Calcium level were estimated by Arsenazo method.

**STATISTICAL ANALYSIS**

Statistical analysis was done using SPSS version 16. The data were reported as mean ± SD, student 't' test used to compare the differences between groups, with P<0.05 considered to be statistically significant.

**RESULTS**

Table 1 shows that Urinary citrate, phosphorous, magnesium levels in controls and cases. Urinary citrate, magnesium, phosphorous were significantly lowered in Urolithiasis patient where as urinary calcium and uric acid levels were significantly higher in Urolithiasis patients compared to control groups.

**Table-1 : 24 Hrs Urinary constituents in first time stone formers and in controls.**

<table>
<thead>
<tr>
<th>Parameters mg/day</th>
<th>Control (Group-I)</th>
<th>Cases (Group-II)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate</td>
<td>739 ± 228.12</td>
<td>271.78 ± 26.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium</td>
<td>116.66 ± 45.74</td>
<td>39.22 ± 4.287</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>688.84 ± 162.6</td>
<td>251.12 ± 29.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>170.92 ± 35.32</td>
<td>291.76 ± 36.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid</td>
<td>591.68 ± 136.79</td>
<td>819.66 ± 40.307</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

24 hours urine volume in cases were significantly lowered when compared to control group. Urine pH in cases (<5.5) was significantly lowered when compared to control group (>6).

Fig-1 shows the comparison of 24 hours urinary citrate level between the control and cases 24 hours urinary citrate in control group 739 ± 228.12 and in cases 271.78 ± 26.72. Citrate in cases were significantly lowered when compared to control group. (p value <0.001).

![Urinary Citrate level](Image)

24 hours urinary Magnesium in control group 116.6 ± 45.74 and in cases 39.22 ± 4.287. Magnesium in cases were significantly lowered compared to control group. (p value <0.001).

24 hours urinary Phosphorus in control group 688.84 ± 162.6 and in cases 251.12 ± 29.4. Phosphorous in cases were significantly lowered compared to control group. (p value <0.001)

24 hours urinary calcium in control group 170.92 ±35.32 and in cases 291.76 ± 36.03. Calcium in cases were significantly higher when compared to control. (p value <0.001).

24 hours urinary uric acid in control group 501.68 ±136.79 and in cases 819.66 ± 40.307. Uric acid in cases were significantly higher when compared to control among the urolithiasis. (p value <0.001)
DISCUSSION

Urolithiasis is a common clinical disorder affecting larger population worldwide and this applies to India as well. Urinary citrate excretion is basically determined by acid-base balance, metabolic acidosis is not always manifest in urinary stone patients with hypocitraturia. Hypocitraturia or low urinary citrate excretion is known as a risk of nephrolithiasis.\(^{(12)}\)

In the present study 24 hour urinary volume, pH, citrate, magnesium and phosphorus were significantly lowered in urolithiasis patients when compared with healthy controls whereas urinary calcium, uric acid, levels were significantly increased in cases compared to control. Frank et al. have found that increase in urine volume was capable of preventing urinary stone formation. A low urine volume is a universal risk factor for all types of kidney stones. A high volume of urine helps to reduce the relative supersaturation of the crystal components. In addition, high volume implies high urine flow rates, which will tend to wash out any crystals that have formed, since poor urine flow effectively increases the concentration of salts and will enhance crystal formation. Urinary stones disease is common pathology encountered in urological practice. Supersaturated urine and its stagnation are well known facts for the development of Urolithiasis. Metabolic disorders like hypercalciuria, hypocitraturia are also responsible for formation of Urolithiasis.\(^{(13)}\)

In the present study the 24 hours urine volume was significantly low in cases when compared to healthy controls. This observation agrees with other studies where lowered urinary volume, pH citrate, magnesium and phosphorus were found in urolithiasis patients. The intratubular citrate protonation depends on ambient pH. The low luminal fluid pH decreases free citrate as it drives the reabsorption of protonated citrate.

In the present study the pH of urine in stone formers was 5 ± 0.05 whereas in control group it was 6.3 ± 0.04. Above pH 6, virtually no urine is supersaturated and below pH 5.3 virtually all are supersaturated. Kok, papapoulos and bijvoet et al. described low urinary citrate excretion accompanied by an abnormally high rate of crystal agglomeration. Approximately 80% of stones are composed of calcium oxalate and calcium phosphate; 10% of struvite (magnesium ammonium phosphate produced during infection with bacteria that possess the enzyme urease), 9% of uric acid and the remaining 1% are composed of cystine or ammonium acid urate or are diagnosed as drug-related stones.\(^{(14)}\)

Citrate is a most potent naturally occurring urinary stone inhibitor and its concentration depends upon the tubular absorption capacity which depends upon blood pH. Thus, a delicate balance is maintained at physiological range of pH of body fluids. Alkalosis increases and acidosis decreases the urinary citrate level. Most therapeutic agents that modify citrate excretion in stone disease usually work by changing pH of tubular fluid.

The Calcium level of urine increases and the corresponding Citrate levels decreased in stone formers. This indicates the loss of balance between citrate and calcium levels. Citrate is a well-known inhibitor of stone crystallization, it chelates calcium in the urine and helps to prevent precipitation of calcium by forming a soluble complex. When citrate excretion is reduced, less calcium is chelated and urolithiasis formation is promoted.

Rizvi et al. have reported malnutrition, chronic dehydration and a diet poor in proteins and rich in oxalates, lead to formation of Ammonium Hydrogen Urate (AHU) stones group as compared to the Calcium Oxalate (CaOx) group while hypercalciuria and hypernatriuria where significantly high in the Calcium Oxalate group as
results in increased urinary calcium level, as sufficient magnesium is not available to form the magnesium - calcium complex. Many research work confirms low urinary magnesium excretion was associated with high urinary calcium which promotes Urolithiasis. Hypocitraturia may be an ominous sign for stone formation and an obvious finding in the present study. The major limitation of this study is its smaller sample size.

CONCLUSION

Hypocitraturia, an easily correctable biochemical abnormality, is common in Urolithiasis patient. A significant decrease in urinary citrate level in renal stone formers compared to healthy controls, suggesting that hypocitraturia is an important metabolic risk factor for renal stone formation.

REFERENCES


