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Research Article

Urinary Citrate excretion in patients with Urolithiasis

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ABSTRACT

Objective: To determine urinary citrate excretion in patients with urolithiasis and normal controls. A prospective study was coducted at ACPM Medical College, Dhule. **Methods:** This study was carried out on, 100 normal individuals and 100 kidney stone patients (76males and 24 females) with idiopathic urinary calculi and 100 ages and weight matched controls. The patients were divided into 3 groups: Group 1 (15-30 years), Group 2 (31-45 years) and Group 3 (46-60 years). Urinary citrate was estimated in a 24-hour urine sample using colorimetric method. The stones removed from these Patients were also analysed. **Results**: There was a preponderance of urinary stones in males; the highest incidence being in Group 2. Excretion of citrate in 24-hour urine sample was significantly lower in patients compared to controls, for males in all age groups and for females. However, there was no statistically significant difference in the urinary citrate value between males and females in a given age group for either controls or patients. The urinary citrate excretion increased with age in patients and controls, but the levels in patients were lower. Depending upon the constituents, four types of stones were identified, calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate. Forty six stones had at least more than one major constituent. Hypocitraturia was detected in 93% cases. The incidence was 72% for calcium phosphate, 89% for calcium oxalate, 36% for uric acid stones and 25% for magnesium ammonium phosphate. **Conclusion:** This study shows that low urinary citrate is associated with urinary stones in patients, especially in endemic areas, in the absence of obvious etiological factors. Urinary citrate excretion should be determined in all patients with urolithiasis.

Keywords: Hypocitraturia, Urinary calculi, Urinary citrate

1. INTRODUCTION

Urinary stone disease continues to occupy an important place in everyday urological practice. The average lifetime risk of stone formation has been reported in the range of 5-10%. There is an incidence peak between the fourth and fifth decade of life. Recurrent stone formation is a common problem with all types of stones and therefore an important part of the medical care of patients with stone disease. The urinary inhibitors of crystal nucleation, growth and aggregation play an important part in urolithiasis. Such inhibitors are presuming to afford protection against stone formation in normal individuals. Citrates and glycosaminoglycans are the most important and almost completely account for urinary inhibiting activity. Citrate is a natural substance that inhibits urinary calcium stone formation. Deficient urinary excretion of citrate has often associated with urinary stone disease. Moreover, successful correction of hypocitraturia, which has been documented largely in adults, positively correlates with a decreased stone recurrence rate [1].

Pathogenic Role of Citrate in Calcium Nephrolithiasis

Citrate retards the crystallization of stone forming calcium salts by two mechanisms:

1. The principal action is the complexation of calcium causing a reduction in ionic calcium concentration and in the urinary saturation of stone-forming calcium salts [2, 3].
2. Citrate directly inhibits the crystallization of calcium oxalate and calcium phosphate by agglomeration of calcium oxalate. It has a modest inhibitory role on the growth of calcium oxalate crystal [4]. Citrate acts as a potent inhibitor of crystal growth of calcium phosphate. In addition citrate has the ability to impair urate induced crystallization of calcium oxalate [5].

The excretion of citrate in the urine is a function of filtration, reabsorption, peritubular transport, and synthesis by the renal tubular cell. The proximal tubule reabsorbs most (70-90%) of the filtered citrate, and citrate secretion is negligible. Acid-base

status plays the most significant role in citrate excretion. Alkalosis enhances citrate excretion, while acidosis decreases it. In acidosis, increased citrate utilization by the mitochondria in the tricarboxylic acid cycle occurs. This results in lower intracellular levels of citrate, facilitating citrate reabsorption and hence reducing citrate excretion. Citrate excretion is impaired by acidosis, hypokalemia (causing intracellular acidosis), high–animal protein diet (with an elevated acid-ash content), and urinary tract infection (UTI).

2. MATERIAL AND METHODS

Twenty four hours of urine samples and blood samples were collected from both patients and controls were analysed from 100 patients with stone disease (76males and 24 females). Renal stone patients were selected among those attending the local clinics at A.C.P.M.Medical College, Dhule, Maharashtra (India). One hundred healthy persons of Dhule district (75 males and 25 females) , who served as controls, with no recent report of ill health of any kind and had no past history of urolithiasis, including that in the family. The diagnosis of urolithiasis was supported by plain abdominal X–ray, ultrasonography and / or intravenous pyelography.

The controls and study patients were from north-Maharashtra, belonged to similar socio-economic status and were matched for age and weight. No dietary restrictions were imposed and the subjects were advised to maintain an optimum fluid intake throughout the study period. The subjects were not on any drug *e.g.*, acetazolamide and thiazides, that could have altered the blood or urinary levels of citrate or calcium. Urine pH was measured and the 24-hour urine estimated for calcium, creatinine, sodium, potassium and uric acid. Blood levels of pH, bicarbonate, calcium, phosphate, uric acid, creatinine, urea, sodium and potassium were measured. For estimation of citrate, the 24-hour urine was collected in a container using 10 ml of 10 N sulphuric acid as preservative. Citric acid estimation was done using the colorimetric method based on oxidation of citric acid in urine to pentabromacetone and the absorbance read at 445 nm [6]. This method was chosen for its sensitivity, feasibility and high reproducibility of results. Urinary citrate levels estimation were expressed as mg/g creatinine. Patients with citrate excretion below 2 standard deviations (SD) were diagnosed as hypocitraturia.

For purposes of comparison, the Patients were divided into 3 groups: Group 1 (15-30 years), Group 2 (31-45 years) and Group 3 (46-60 years), for either sex. The distribution of the number of subject in each of these groups is shown in Table 1. The stones removed were dried, powdered and examined chemically.

Statistical Methods

Mean values and SD was calculated for each of the three age groups. Chi-square test was applied to compute the significance of the categorical variables; $p < .05$ was considered marginally significant and $p < 0.01$ was considered significant.

3. RESULTS

There was an overall preponderance of urinary stones in males; the highest incidence of urolithiasis was seen between 15 to 45 years of age (group 1 & 2) (Table 1). We found all study patients to have a relatively higher excretion of calcium as compared to controls, the difference was statistically significant. Excretion of citrate in 24-hour urine sample was significantly lower in patients as compared to controls, for males in all Groups. Females in Group 2 also showed a significantly lower citrate excretion, though those in Groups 1 and 3 showed only a marginally lower citrate. There was no significant difference in the urinary citrate excretion between males and females in any Group. The urinary citrate excretion versus age showed similar pattern for patients and controls (an increase in citrate excretion with increasing age), but the levels in stone formers were lower (Fig. 1).

Table 1-Weight distribution and citrate excretion (mean ± SD) among patients and controls

	Controls (n = 100)		**Patients (n = 100)**	
	Male (n = 75)	**Female(n = 25)**	**Male (n = 76)**	**Female (n = 24)**
Group 1 (15 - 30 years)	29	05	36	05
Group 2 (31 - 45 years)	35	12	33	08
Group 3 (46 - 60 years)	11	08	07	11
Weight (kg)				
Group 1	68.2 ± 5.6	54.8 ± 6.6	71.6 ± 4.6	55.6 ± 2.5
Group 2	76.3 ± 6.2	67.0 ± 5.3	79.6 ± 5.5	72.0 ± 1.6
Group 3	62.6 ± 4.8	53.8 ± 4.9	61.1 ± 2.9	53.7 ± 3.3
24-hour citrate excretion mg/ g creatinine				
Group 1	234.4 ± 39.9	228.9 ± 63.9	107.6 ± 47.5*	92.0 ± 41.1*
Group 2	239.7 ± 43.7	207.4 ± 68.7	84.5 ± 44.8*	78.6 ± 28.6*
Group 3	241.2 ± 32.1	192.9 ± 54.2	87.8 ± 53.7*	84.9± 15.6*

** Statistically significant lower excretion of citrate compared to controls ($p < 0.01$)*

 # No statistically significant difference between males and females in any age group for patients or controls

Depending upon the constituents, four main type of stones were identified namely, calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate. Of 100, 46 stones had at least more than one major constituent. Table 2 shows the distribution of these stones and the mean level of urinary citrate in different stones.

Table 2: Citrate Excretion (mean ± SD) in Major Stones (n = 100)

Type of stone	Citrate Excretion (mg/g creatinine)
Calcium phosphate (n = 41)	131.0 ± 42.0
Calcium oxalate (n = 31)	114.4 ± 29.2
Uric acid (n = 24)	139.5 ± 33.4
Magnesium ammonium phosphate (n = 4)	142.0 ± 34.5

46 stones had more than one component

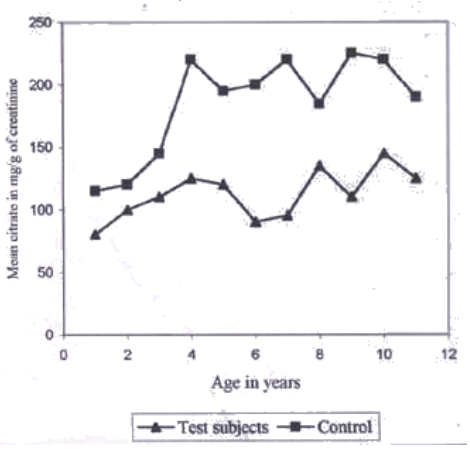

Fig. 1: Agewise distribution of mean urinary citrate levels (mg/g creatinine) in controls and study subjects

The Group 2 and Group 3 showed statistically higher levels of urinary uric acid. Since the serum uric acid was within normal limits and there were no features of disturbed purine metabolism, this finding was believed to be consistent with the observation that idiopathic calcium oxalate stone formers generally have a higher uric acid excretion.

Hypocitraturia was detected in 93% cases. The incidence was 72% for calcium phosphate, 89% for calcium oxalate, 36% for uric acid stones and 25% for magnesium ammonium phosphate stones.

4. DISCUSSION

Citric acid is an important intermediate in metabolism. In humans, citrate is both metabolized and excreted by the kidney and its presence in urine contributes to the inhibitory potential against crystallization of calcium salt: stone formers show significantly lower mean values of citric acid excretion and urine concentration than do normal subjects. Citrate acts both through surface controlled mechanisms to hamper crystal growth and aggregation and through the formation of stable soluble complexes with calcium. Therefore, citrate determination has become an important tool in the assessment of urine supersaturation with respect to calcium oxalate and phosphates [7].

Citric acid is the strongest complexing agent for calcium in urine. It plays an important role as an "Inhibitor" in preventing supersaturation with respect to the formation of calcium oxalate. The diminished excretion and even more the low concentration of citric acid in 24-hour urine specimens of stone formers are found by all investigators [8]. Healthy controls excrete significantly more citric acid in 24-hour urine samples than stone formers.

The urinary citrate levels are infact subject to wide variations and depend to some extent on dietary habits. Larger intake of animal protein and sodium generally reduces the urinary citrate level. Presently we observed servere hypocitraturia in almost all stone patients. This might be because all these patients are nonvegetarians and are in the habit of eating non-vegetarian food frequently [9].

Since citrate excretion increases with increase in body weight, urinary citrate excretion in relation to creatinine gives a better assessment of stone risk [10]. Using this method, the normal values for urinary citrate excretion for the Indian male were found to be 385.1 ± 41.1 mg/g of creatinine. In the present study, the values for citrate among normal and stone forming patients were computed based on age and sex. We found significantly lower urinary citrate excretion in stone formers in all Groups for males and for females in age group. In our study we found dominance of urolithiasis in males. The average per day citrate excretion was found to be lower in stone formers. This is significantly lower than the values for control. Citrate chelates calcium in the urine, helping to prevent precipitation of calcium salts, particularly in alkaline urine. Under normal conditions as much as 70% of the calcium in the urine may be bound to citrate when citrate excretion is reduced, less calcium is chelated and nephrolithiasis formation is promoted.

In summary, hypocitraturia (low urine citrate excretion) 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.

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