Original Research Article

A study on evaluation of different urinary constituents and their ratios in patients with urolithiasis

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Abstract

Background

The lifetime prevalence of kidney stone disease is estimated 1% to 15 % with the probability of varying according to age, gender, race and geographic location. Adult men are 3 times more affected than adult women. Calcium oxalate stones are common in south east. Uric acid stones are common in the east. In India prevalence is more than 11%.Stone disease peaks at 4th to 6th decade. Higher prevalence of stone disease is found in hot, arid or dry climates. Metabolic defects are less likely to occur in the first time stone formers than in patients with recurrent disease. Hypocitraturia and hyperoxaluria are common abnormalities found in stone formers. Stone formers have significantly higher calcium, oxalate and uric acid levels. Citrate/calcium ratios are found to be low in stone formers.**Objectives**:1.Estimation of urinary parameters such as calcium, magnesium, uric acid, creatinine, oxalate, phosphate and citrate in 24 hour urine. 2.Estimation of calcium/creatinine, citrate/creatinine, uric acid/creatinine, calcium/citrate ratios in 24 hour urine and specifically to screen a patient with renal stone and in turn it will help in the treatment and prevention of stone formation in recurrent stone formers.**Materials &Methods**:The study included 100 subjects comprising of 50 healthy controls and 50 urolithiasis cases. 24 hour urine was collected and toluene was added as preservative. Urine calcium was estimated by Arsezano's method. Urine magnesium was estimated by Xylidyl blue method. Urine phosphorus was estimated by end point method. Urine uric acid was measured by uricase method. Urine creatinine was estimated by modified Jaffe's method. Citrate and oxalate was estimated by enzymatic method.**Results**:The urinary calcium, phosphorus, uric acid, oxalate was increased in cases when compared to controls. Urine magnesium and citrate was reduced when compared to controls. Urine creatinine was normal or decreased in cases than controls.**Conclusion**:This study is being undertaken to estimate different ratios so as to get specific ratio as an index in stone formers whereas high calcium/creatinine ratio can be used as a screening procedure to detect increase calcium in urine.

Keywords: Urolithiasis, calcium, magnesium, uric acid, phosphorus, creatinine, citrate, oxalate.

Introduction

Urolithiasis is one of the most common diseases of the urinary tract which has tormented people throughout the ages. The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing. Urinary stone formation tends to recur at a very high rate without preventive measures after a first stone. Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened[1].Most kidney stones are predominantly composed of calcium oxalate[2].Despite enormous developments in nephrology and urology; we still do not know exactly how kidney stones are formed and how to prevent them[3].

Most kidney stones are predominantly composed of calcium oxalate.Despite enormous developments in nephrology and urology;we still do not know exactly how kidney stones are formed and how to prevent them.Urolithiasis denotes stones originating anywhere in the urinary tract, including kidneys, ureters and bladder[4].Stones are built from numerous tiny crystals that commonly are pasted together with organic material. The formation of crystals in the kidney is normal and harmless provided that they are excreted with the urine. The difference between stone formers and non–stone formers is that crystals stay behind in kidneys of stone formers[1].Factors leading to initiation of calcium oxalate stone formation are still not known.[5] Experiments performed on animals, cultures and human sera have revealed that there is presence of enhanced oxidative stress in stone forming conditions[6].Crystal adherence to the surface of injured renal epithelial cells is considered as initiating event in the genesis of urolithiasis. Surgical intervention removes only the stones but not their cause and also do not avoid the possibility of new stone formation. So there is a need for alternative management of urolithiasis[7] effective therapeutic approach.

Objectives

1. Estimation of urinary parameters such as calcium, magnesium, uric acid, creatinine, oxalate, phosphate and citrate in 24 hour urine.
2. Estimation of Calcium/creatinine, Uricacid/creatinine& Citrate/calcium ratios in 24 hour urine of urolithiasis patients and healthy controls.
3. Toevaluate Calcium/creatinine, Uricacid/creatinine& Citrate/ calcium ratios as a screening test for urolithiasis.

Methodology

A case control study of urinary calcium, magnesium, phosphorus, uric acid, creatinine, citrate and oxalate and their ratios in urolithiasis patients and in healthy controls were carried out from April 2019 to April 2020.

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Controls and urolithiasis disease cases were selected from MGM Hospital (both these hospitals are attached to teaching institute,Kakatiya Medical College, Warangal). Each study subject gave an informed consent and this study was approved by the ethical and research committee of Kakatiya Medical College, Warangal to use human subjects in the research study. The patients and controls voluntarily participated in the study.

Subjects:A total number of 100 subjects were selected for the present study based on the inclusion and exclusion criteria. Out of the 100 subjects, 50 were urolithiasis disease patients and 50 were age and sex matched healthy controls.

Inclusion Criteria

Cases: 50 patients with urolithiasis aged 15-80 years diagnosed and confirmed by urologist with either x ray KUB, ultrasonographyof abdomen, computed tomographic scan of the abdomen, intravenous pyelography and in whom routine investigations have been done were included.

Controls: Age and sex matched healthy individuals free from history of smoking, alcoholism and coexistence of any disease like diabetes mellitus, hypertension were included.

Exclusion Criteria

- Patients with history of bowel disease.
- Patients with history of Renal Tubular Acidosis.
- Patients with history of urinary tract anomalies.
- Patients with history of gout.
- Patients with history of diabetes mellitus and hypertension.

Collection of urine samples

24 hour urine will be collected after giving proper instructions to the patients so that there is no room for ambiguity. The urine sample is stored at < 4^0c with adding preservatives like toluene and centrifuged at 100 rpm per minute.

Parameters Measured

The following parameters were estimated in the present study

1.Urinary Calcium
2.Urinary Magnesium
3.Urinary Citrate
4.Urinary Oxalate
5.Urinary Creatinine
6.Urinary Phosphorus
7.Urinary Uric acid

Units	Reference range
Urinaru Calcium	100-300 mg/day of Urine
Urinary Megnisium	75 - 125 mg/24h of Urine
Urinary Phosphorus	400-500 mg/day of Urine
Urinary Uric acid-Urine	250-750 mg
Urinary Creatinine	1.0 -2.0 mg/day
Urinary Citrate	< 350 mg/day in males &<500 mg/day in female

Results

Table 2: showing age and sex wise distribution of subjects

		Controls	Cases
Number of subjects		50	50
Age(years)	Mean± S.D	39.66±16.91	43.88±18.21
	Range	15-67	6-75
Gender	Male	35	39
	Female	15	11

A total number of 100 subjects were included in this study. Among them 50 were controls who were normal healthy individuals and 50 were urolithiasis cases. Out of 50 controls 35 were males and 15 were females, the mean of the age being 39.66±16.91years. Among 50 urolithiasis cases 39 were males and 11 were females, the mean age being 43.88±18.21.

Table 3:showing the various parameters their mean, t value, p value and standard deviation

Parameters		Controls (n=50)	Cases (n=50)	Cases/Controls		
				Mean difference	t ** value	p value
U. Calcium	Range	190-270	250-450	4.22	18.59	<0.001*
	Mean ± SD	237.1±21.5	358.8±40.9			
U.Magnesium	Range	77-106	65-100	121.70	11.84	<0.001*
	Mean ± SD	89.8±8.3	74.8±5.3			
U.Phosphorus	Range	300-370	300-630	169.38	10.62	<0.001*
	Mean ± SD	342.6±20.62	512.3±99.08			
U. Uric Acid	Range	312-421	300-800	14.96	9.96	<0.001*
	Mean ± SD	374.5±29.92	588.7±148.8			
U. Creatinine	Range	800-910	600-920	213.88	3.72	<0.001*
	Mean ± SD	857.5±43.60	813.4±71.94			
U. Citrate	Range	354-480	116-290	44.22	15.94	<0.001*
	Mean ± SD	380.34±53.7	216.5±44.06			
U. Oxalate	Range	35-45	36-102	157.30	9.18	<0.001*
	Mean ± SD	40.14±2.69	69.4±22.38			
U. Calcium/ Creatinine	Range	0.205-0.329	0.275-0.663	29.26	16.28	<0.001*
	Mean ± SD	0.277±0.027	0.444±0.067			
U. Uric Acid/ Creatinine	Range	0.357-0.512	0.427-1.333	.167	9.36	<0.001*
	Mean ± SD	0.437±0.037	0.733±0.330			
U. Calcium/ Citrate	Range	0.488-6.324	0.992-2.586	.295	7.76	<0.001*
	Mean ± SD	0.626±.808	1.72±0.396			

The above table shows the comparative analysis of urinary calcium, magnesium, phosphorus, uric acid, creatinine, citrate, oxalate, calcium/creatinine ratio, uric acid/creatinine ratio and calcium/citrate ratio in controls and cases of urolithiasis.

The mean values of urinary calcium, magnesium, phosphorus, uric acid, creatinine, citrate, oxalate, calcium/creatinine ratio, uric acid/creatinine ratio and calcium/citrate ratios in controls are 237.1±21.51, 89.8±8.3, 342.6±20.62, 374.5±29.92, 857.5±43.60, 380.4±53.7, 40.14±2.69, 0.277±0.027, 0.437±0.037, 0.626±.808 respectively.The mean values of urinary calcium, magnesium, phosphorus, uric acid, creatinine, citrate, oxalate, calcium/creatinine ratio, uric acid/creatinine ratio and calcium/citrate ratios in cases are 358.8±40.9, 74.8±5.3, 512.3±99.08, 588.7±148.8, 813.4±71.94, 216.5±44.06, 69.4±22.38, 0.444±0.067, 0.733±0.330, 1.72±0.396

respectively.Statistical analysis by unpaired t test showed that urinary calcium, phosphorus, uric acid and oxalate were significantly increased value (p value<0.001) whereas urinary magnesium, citrate and creatinine were decreased.Receive Operative Curve analysis (ROC) was done to assess the diagnostic accuracy of the calcium/creatinine, uric acid/ creatinine and calcium/citrate ratio and was significant.(p value<0.05).

Table 4:ROC showing the significance of the ratios

Parameter	AOC	p value
Cal/creatinine ratio	.991	<0.05*
Uric acid/creatinine ratio	.945	<0.05*
Calcium/citrate ratio	.980	<0.05*

The ROC shows that the AUC of the Calcium/creatinine ratio, Uric acid/ creatinine ratio, and Calcium/citrate ratios are 0.991, 0.945, 0.90 respectively, so the ratios have diagnostic value.(p value<0.05).

Table 5:showing the sensitivity and specificity of the ratios

	Cal/creat	UA/Creat	Cal/citrate
Cutoff value	0.33	0.53	0.85
Sensitivity	98%	76%	100%
Specificity	100%	100%	100%
Positive Predictive Value	100%	100%	100%
Negative Predictive Value	98%	80%	100%

The above table shows the specificity and sensitivity of the urinary ratios.

Discussion

Urolithiasis denotes stones originating anywhere in the urinary tract, including the kidneys, ureters and bladder[4].Urinary calculi are the third most common affliction of the urinary tract, constituting an important part of everyday urological practice[8]. Recurrent stone formation is a common part of the medical care of patients with stone disease[3].Kidney stones cause considerable suffering and have a substantial economic impact. Although it is perceived as an acute illness, stone disease is now gradually accepted as a chronic systemic disease that may lead to renal loss[10].It is a common chronic disorder affecting 10-15% of population worldwide and 11% of population in India[9].The lifetime risk for kidney stone disease exceeds 7- 12% in the general population and the prevalence of upper tract stone disease has been reported to be increasing[10].The occurrence of urolithiasis in men is two to three times higher than in women[4].Severe pain or aching in the back on one or both sides, sudden spasms of excruciating pain (renal or uteric colic), bloody, cloudy urine, feeling of being sick, a frequent urge to urinate, or a burning sensation during urination, fever and chills etc are commonly observed symptoms in the patients[4].Stone prevention is important due to recurrence rates without medical treatment of more than 50% over 10 years. Knowledge of the underlying mechanism of stone formation can potentially lead to novel therapies targeting the formation process, enhancing prevention efforts[10,11].The main aim is to evaluate the urinary parameters and their ratios in patients of urolithoiasis.The present study includes 100 subjects of which 50 were urolithiasis patients and 50 were normal healthy individuals.

The mean values of urinary calcium, magnesium, phosphorus, uric acid, creatinine, citrate, oxalate, calcium/creatinine ratio, uric acid/creatinine ratio and calcium/citrate ratios in controls are 237.1±21.51, 89.8±8.3, 342.6±20.62,374.5±29.92, 857.5±43.60, 373.4±53.7, 40.14±2.69, 0.277±0.027, 0.437±0.037, 0.739±.808 respectively.The mean values of urinary calcium, magnesium, phosphorus, uric acid, creatinine, citrate, oxalate, calcium/creatinine ratio, uric acid/creatinine ratio and calcium/citrate ratios in cases are 358.8±40.9,74.8±5.3,512.3±99.08,588.7±148.8,813.4±71.94, 216.5±44.06, 69.4±22.38, 0.444±0.067, 0.733±0.330, 1.72±0.396 respectively.

Urinary Calcium & Phosphorus

Hypercalciuria is the excretion of urinary calcium more than 4mg/kg/d while ingesting routine diet. In our study calcium was increased in cases than controls because it is a stone promoter and stone formation results from imbalance between promoter and inhibitor. So, renal hypercalciuria is a major determinant of stone disease. Serum calcium level remains normal because the renal loss of calcium is compensated by enhanced intestinal absorption of calcium and bone resorption as a result of increased secretion of parathyroid hormone and enhanced synthesis of calcitriol [$1,25(OH)_2D_3$)]. The calcium stone formation largely depends on calcium concentration rather than others. Calcium stones are opaque. Phosphorus levels are generally increased in calcium phosphate stones and mixed stones but remains normal in other kinds of stones. In our study urinary phosphorus levels were more in cases than controls. So this predicts that the patients in the study group had either calcium phosphate or mixed stones.This study is in accordance with study of Srinivas S[11]

Urinary Magnesium

Magnesium is an important element in biological calcification process. It may act as a promoter in triple phosphate stone and inhibitor in calcium phosphate and calcium oxalate crystallization. In our study magnesium was decreased in cases than controls, that is, it acts as an inhibitor predicting calcium oxalate and uric acid stones. In reference to calcium oxalate, magnesium is seen to inhibit because there is Mg- induced decrease in both biosynthesis and urinary excretion of oxalate, formation of complex. Calcium oxalate calculi growth is retarded. Magnesium induced glomerular injury is common in recurrent calcium urolithiasis. Cellular Mg deficiency would lead to impaired phosphorylation due to uncoupling of Mg substrate binding. Another important hypothesis is magnesium acts as stone inhibitor in acidic pH and promoter in alkaline pH.This study is in accordance to Angelika Schmiedl[12].

Urinary Oxalate

Oxalate is a major promoter of urinary calculi formation. Hyperoxaluria is a major predisposing factor than hypercalciuria and hypocitraturia. Oxalate rich diet can lead to calcium oxalate stone formation. The patho physiology of kidney stones is the formation of crystals in the tubular fluid or urine. Nuclei formation occurs when stone forming elements reach their upper saturation limits. Calcium oxalate is the most predominant type of calculi. Most of the oxalate is a metabolic end product generated in the liver. Important oxalate sources such as chocolates, tea, cinnamon and turmeric. The bulk of oxalate absorption occurs in proximal convoluted tubule. The difference in oxalate absorption is due to the action of oxalate degrading bacteria oxalobacterformigenes. The only oxalate transporter in the intestine is CFEX (chloride formate exchanger). These findings are in accordance to a study by Hrvoje Brazica[8].

Urinary Citrate

The level of citrate is an important factor in the control of calcium urolithiasis. It is a potent inhibitor of urolithiasis. In our study citrate is decreased in cases than controls because the capacity of the citrate which chelates the calcium is lost as hypercalciuria occurs. The mechanism of inhibitory action of citrate is probably through the

chelating of calcium ions in urine and it also prevents combining calcium with stone forming anions. Citrate taken with calcium oxalate enhances oxalate excretion. Citrate plays an important role in calcium urolithiasis by enhancing ascorbate induced oxalogenesis. It also increases absorption and excretion of calcium. These findings co relate to a study by TVRK Rao[13].

Urinary Creatinine

Creatinine is an anhydride of creatine produced by methionine, glycine and arginine. Creatinine is an indicator of glomerular functioning of the kidney because it is neither secreted nor metabolized in our body. Creatinine may not be regarded as an inhibitor but it is important to calculate creatinine levels for urinary ratios because its excretion remains constant in 24 hours. In our study, creatinine is decreased in urolithiasis cases than controls and thus can be regarded as an inhibitory substance in the near future with the support of other studies. This study is in accordance with the study of M.R Willis[14].

Urinary Uric Acid

Uric acid stones compose <5% of all urinary calculi and are usually found in men. Patients with gout, myeloproliferative diseases, rapid weight loss and those treated for malignant conditions with cytotoxic drugs have a high incidence of uric acid lithiasis. Most patients with uric acid calculi do not have hyperuricemia. Elevated uric acid levels are frequently due to dehydration and excessive purine intake. Hyperuricosuria is defined as urinary uric acid exceeding 600 mg/day. Up to 10% of calcium stone formers have high urinary uric acid levels as the only abnormality. Hyperuricosuria increases urinary levels of monosodium urate, which in turn promotes calcium oxalate stone formation. At pH less than 5.5, the undissociated form of uric acid predominates, leading to uric acid and/or calcium oxalate stone formation.In our study Calcium/creatinine ratio, Uric acid/creatinine ratio, Calcium/citrate ratio is increased because of the increase in stone forming substances such as calcium and uric acid and decrease in citrate, inhibiting substance. These ratios are very sensitive and important because it can specifically tell the promoter which has increased and help in recurrent stone formers. In the near future these ratios are going to replace the standard parameters because they are more sensitive and specific as per our study. Calcium oxalate is the most common type of stone in our perspective.Hypercalciuria and hyperuricosuria seemed to be the most important metabolic factors of calculus forming in our pediatric series.We conclude that the dietary components influence the biochemical parameters such as oxalate, uric acid, calcium. Accordingly, quantitative as well as qualitative dietary modifications especially for oxalate, animal protein, and minerals may play an important role in reducing the likelihood of recurrent stone formation. Dietary modifications could play an important part in the management of stone disease in the region. We should keep in perspective the social and cultural environment of the stone formers. The dietary modifications should be advised in the long term.Our results show that in our population hypocitraturia along with hyperoxaluria and hypercalciuria are the major contributory factors in stone formation. Hypocitraturia coexisting with hypercalciuria in recurrent stone formers is an interesting observation that should be further investigated in a more heterogeneous population. Emphasis should be in the form of dietary restrictions or counselling to the patients to enhance stone inhibitors like citrate to prevent recurrence of stone formation[14,15,16,8]

Conclusion

From our study we see that there is highest prevalence of calcium oxalate stone followed by uric acid stone. Our study illustrates an extensive biochemical investigation in patients with urolithiasis in order to avoid recurrence and potential progression towards CRF. Dietary and environmental factors play an important role in stone formation. The promoters, inhibitors and predisposing factors contribute to stone formation. It was seen that hypocitraturia, hypercalciuria and hyperoxaluria are major predisposing factors in stone formation. High calcium/creatinine ratio and uric acid /creatinine ratio was used as a screening procedure to detect hypercalciuria and hyperuricosuria. Recent studies also show that a primary interstitial apatite crystal formation (Randal Plaque) secondarily leads to Calcium Oxalate stone formation. So this study was undertaken to estimate different ratios so as to get specific ratio as an index in stone formers so that dietary restrictions can be and treatment can be given to the recurrent stone formers accordingly.

Conflict of Interest: Nil Source of support:Nil

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