

Original article:

24 hours urinary analysis for renal stones promoters and inhibitors in North India

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Abstract:

Nephrolithiasis is a worldwide problem, occurs both in men and women with male preponderance. Average ages of presentation 30–40 years in male, and somewhat later in women. Kidney stones are typically classified by their location and chemical composition. Urinary stone usually arises because of breakdown of delicate balance of solute in urine. In our study gives an incling that hypocitraturia present in 73.33% cases is by far the biggest risk factor followed by hypercalciuria, with mild hypercalciuria present (calcium excretion between 200-250mg/day) in 43.33% cases, moderate hypercalciuria present (calcium excretion between 250-300mg/day) in 23.33% cases and severe hypercalciuria (calcium excretion >300mg/day) in 16.66% cases. The oxalate excretion varies in selected population and mild hyperoxaluria (oxalate excretion >40mg/day) present in 23.33% (n=7) cases and moderate hyperoxaluria (oxalate excretion >50mg/day) present in 3.33% (n=1) case only Normal urinary excretion of uric acid in all patients. Needless to say that that the selected healthy individuals (controls) shows excretion of promoters and inhibitors in urine within normal limits except mild hypercalciuria was present in 9(30%) cases and mild hypocitraturia present in 4(13.33%) cases. and THP level was on higher side in 2(6.66%) healthy individuals only.

Key words: Renal Stones, Urinary Analysis, Promoters and Inhibitors

Introduction

Nephrolithiasis is a worldwide problem, sparing no geographical, cultural, or racial groups. It occurs both in men and women but the risk is generally high in men between ages of 30–40 years, while for women the age at first presentation is somewhat later. This difference in prevalence in males and female might be due to the presence of estrogen, which protects women against kidney stone formation.^[1] Estrogens may also help to prevent the formation of calcium stones by keeping urine alkaline and raising protective citrate levels.^[2] One of the reasons for increased incidence in males is attributed to increased dietary protein intake, which increases urinary excretion of calcium, phosphates and magnesium and reduces urinary citrate

concentration. The lifetime risk is about 10–15% in the developed world, but can be as high as 20–25% in the Asian countries.

Kidney stones are typically classified by their location (upper or lower urinary tract) and chemical composition. On the basis of chemical composition the stones are mainly of four types, Calcium containing stones (70-75 %), Struvite or triple stones (10-15%), Uric acid stone (5-10%), Cystine stones (1-2%) and others. The incidence of calcium containing stones is very high and occurrence of uric acid stone is far less, and those of other types stones is usually rare e.g. Xanthine and Cystine stones.

Urinary stone usually arises because of breakdown of delicate balance of solute in urine.

The kidney must conserve water, but they must excrete materials of low solubility. Human urine contains stone forming constituents and has potential to induce spontaneous mineralization. In urine stone promoters are high level of calcium, oxalate, uric acid, cystine, xanthine and high level of mucoproteins. Stone inhibitors are mainly citrate and magnesium. Tamm–Horsfall Protein (THP) has been reported to behave both as promoter and inhibitor depending on urinary pH, ionic strength and chemical milieu. When the urine is supersaturated with insoluble materials, because excretion rates are excessive and / or because water conservation is extreme, crystals form and may grow and aggregate to form a stone.

In India calcium and oxalate are the main constituents of renal stones. Beside, this negative role played by calcium, this mineral plays an essential role in cell signaling processes, bone formation and maintenance of homeostasis. Oxalate for a long time was presumed to be inert and undesirable metabolite playing a notorious role in birth and growth of stones. Phosphate is a benign component of urine but with persistent infection in alkaline urine may give rise to calcium phosphate stones. Uric acid is an end product of purine metabolism is a waste product and excreted in urine. Uric acid stones are formed either due to large intake of non-vegetarian diet or with mild genetic predisposition. Protein molecules either alone or in combination with carbohydrate (glycoprotein) exerts inhibitory or promotory influence. Among all macromolecules THP (Tamm Horsfall Protein) has received maximum attention

because in normal excretion it behaves as inhibitor and interestingly when excreted in excess behaves as potent promoter.

One of the potent inhibitor of stone formation in urine is citrate and it is particularly effective against the calcium oxalate and phosphate stones lastly, urinary pH can provide possible incling of the composition of stones embedded in urinary tract. Further on abnormal urinary pH is another risk factor for nephrolithiasis. High urinary pH leads to increased saturation of calcium phosphate predisposing to nephrolithiasis. A low urinary pH predisposes to uric acid nephrolithiasis.

Material and Method

This study was carried out at Eras Lucknow Medical College & Hospital Lucknow in the department of Biochemistry and Surgery. Total 30 cases (patient) and 30 controls were chosen for this study. Only male patients above 18 year were included in this study. The patient having other associated disease which could influence the stone risk factors and those with congenital and other anatomical defects in urinary tract were excluded from the study.

Detailed Urinary parameters physical (routine and microscopy), as well as 24 hours urine samples were examined for stone inhibitors and promoters. In stone promoters calcium, oxalate, uric acid and phosphate and in stone inhibitors citrate and THP were analyzed. All tests were done by standard method. These investigations were performed in all the thirty cases and thirty controls in their fasting urine samples.

Results:

Table 1: 24 hour urinary biochemical analysis of promoter and inhibitors in cases.

S. No.	Age-Group	Cases	Urinary Volume	Calcium	Oxalate	PO ₄	Uric acid	Mg	Citrate	THP
1	20-30	3	1.8 ± 0.17	236 ± 58.71	38.97 ± 9.74	461.43 ± 91.23	460.97 ± 192.1	70.14 ± 8.1	275.33 ± 16.91	42.07 ± 3.73
2	31-40	14	1.73 ± 0.196	228.55 ± 54.08	43.2 ± 4.9	528.99 ± 150.22	401.87 ± 88.9	73.44 ± 5.3	301.69 ± 32.04	35.94 ± 5.72
3	41-50	12	1.73 ± 0.168	221.03 ± 59.17	32.47 ± 7.47	452.88 ± 119.96	429.23 ± 116.4	74.01 ± 5.6	272.2 ± 39.4	37.92 ± 4.997
4	51	1	1.9 ± 0.0	228.8 ± 0.0	29.9 ± 0.0	433.8 ± 0.0	420.6 ± 0.0	82.1 ± 0.0	323.8 ± 0.0	32.9 ± 0.0
5	Overall Mean		1.74 ± 0.18	241.6 ± 57	35.46 ± 8.96	488.6 ± 132.8	419.4 ± 107.7	73.6 ± 5.7	287.2 ± 36.8	37.2 ± 5.4

Table 2: 24 hour urinary biochemical parameters of promoters and inhibitors in controls.

S. No.	Age-Group	Cases	Urinary Volume	Calcium	Oxalate	PO ₄	Uric acid	Mg	Citrate	THP
1	20-30	4	1.73 ± 0.19	218.45 ± 38.09	23.77 ± 3.0	437.2 ± 138.5	292.5 ± 38.3	84.3 ± 26.1	515.1 ± 28	43.7 ± 3.5
2	31-40	13	1.81 ± 0.173	191.28 ± 23.72	20.9 ± 3.17	423.4 ± 106.5	363.9 ± 58.8	86.1 ± 22.9	533.1 ± 155.5	44.5 ± 12.1
3	41-50	11	1.85 ± 0.28	182.05 ± 20.76	20.52 ± 2.99	411.6 ± 103.2	336.9 ± 54.4	76.2 ± 24.4	546.4 ± 191.1	45.6 ± 11
4	> 51	2	1.8 ± 0.14	194.2 ± 9.4	18.3 ± 0.71	350.5 ± 8.1	374.1 ± 70	73.7 ± 7.4	529.6 ± 339.1	36.6 ± 9.4
5	Overall mean		1.81 ± 0.21	192.5 ± 25.9	20.51 ± 3.1	421.2 ± 101.4	348.8 ± 55.7	82.6 ± 22.2	550 ± 180.2	44.4 ± 10.8

Table 3: Values for Urinary Chemistry Tests in 24 hour urine (mean ± stdev)

Features	Age	Volume	Calcium	Oxalate	PO ₄	Uric acid	Mg	Citrate	THP
Normal range		0.8-2lt	25 – 300 mg/	7- 44mg	400- 1300mg	250- 750mg	24- 255mg	116- 926mg	20- 70mg
Cases (n=30)	39.03 ± 6.74	1.74 ± 0.18	241.6 ± 57	35.46 ± 8.96	488.6 ± 132.8	419.4 ± 107.7	73.6 ± 5.7	287.2 ± 36.8	37.2 ± 5.4
Controls (n=30)	38.23 ± 7.03	1.81 ± 0.21	192.5 ± 25.9	20.51 ± 3.1	421.2 ± 101.4	348.8 ± 55.7	82.6 ± 22.2	550 ± 180.2	44.4 ± 10.8
P value	0.655	0.174	0.000	0.000	0.022	0.002	0.075	0.000	0.002

Diagram 1: 24 hour urinary analysis of promoters and inhibitors in Cases.

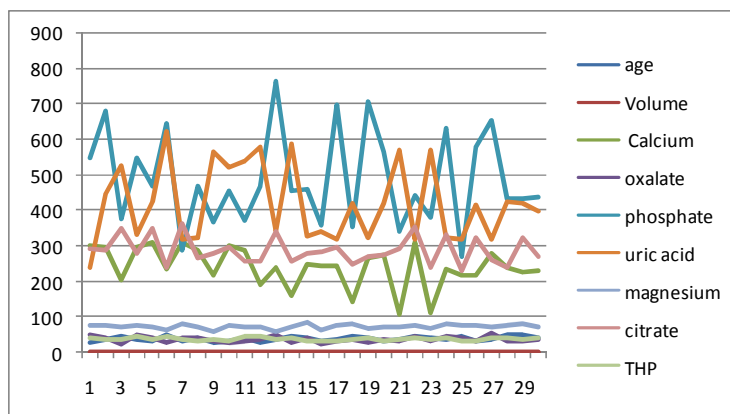
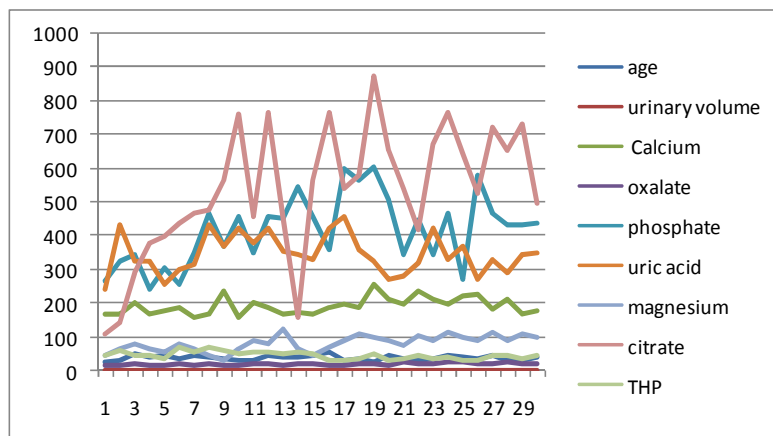


Diagram 2: 24 hour urinary analysis of promoters and inhibitors in control.



Observation & Discussion

Our observation of 24 hours urinalysis results of 30 cases and 30 controls are depicted in table 1 and table 2 respectively. Table 3 consists of mean and standard deviations in compare with the reference value and range and its significance. Figure 1 & 2 shows the graphical presentation of urinary parameters of 24 hour urinalysis of cases and controls respectively.

The disease affected all age groups from less than 1 year old to more than 70years. The incidence of formation first kidney stone between the ages of thirty and seventy vary between approximately 100 – 300 per 100,000 per year in men i.e.6%–9% in males.^[3] In our study we includes the patients above the age of 18 years, and we find that maximum number of cases i.e. 14 out of 30 cases (46.67%) occurs in 31 to 40 age group (4th decade of life), followed by 12 cases (40%) in 41-50 age group (5th decade of life) and 3 cases (10%) in 20-30 age group (3rd decade of life). The only one case (3.33%) was present in age group 51 and above with overall mean age of patients was 39.03 ± 6.74. Our study finding of mean age of the patients corresponds with the study Julka et al. (2012), they has observed similar finding with mean age 38 ±

10.2 years.^[4] In another study by *Kumar et al.* also have the similar finding in their study with mean age of patient was 39 years.^[5] Similar observations were also observed by various other authors in their studies.^[6] Contrary to these study by *Baker et al.* reported that the peak age for the development of calcium oxalate stones was between 50–60 years.^[7] In men, the incidence of kidney stones declines markedly after 60 years of age, suggesting that the pathophysiology of nephrolithiasis is different in the elderly.^[8]

We find in our study that calcium, oxalate, phosphate crystals was present in both acidic and alkaline urine, phosphate crystals more commonly present in alkaline urine. The presence of protein in few cases in urine was not unexpected as nephrolithiasis is likely to produce tissue damage resulting in excretion of protein in urine. Presence of sugar in urine cannot be considered to be a contributory factor for nephrolithiasis. Pus cells are present in few cases bur urinary tract infection was ruled out as urine culture and sensitivity report was sterile in all cases.

The definition of hypercalciuria is an arbitrary one. In western population it is defined as an excretion of >300mg/24 hrs in males. But in

developing countries like India and adjacent countries it is taken as excretion of $>200\text{mg}/24\text{ hrs}$ because of difference in intake of calcium.^[9] We find that 63.33% cases showing hypercalciuria with calcium level of $>200\text{mg}/\text{day}$ and this finding corresponds with the study of *Julka et al.* who shows hypercalciuria in 52% cases.^[4]

In our cases we found mean calcium level were in the range of 228 ± 0.0 to 236 ± 58.71 . Study by *Sharda, et al.* in which mean calcium in 24 hrs urine was 182.4 ± 8.726 . (n=100).^[10] *Gyawali* shows that mean urinary calcium in cases was $161.4\pm 82.3\text{mg}/24\text{ hours}$.^[11] This variation in calcium excretion seems to be due to difference in dietary intake of selected patients. Urinary oxalate is far more potent risk factor than urinary calcium because oxalic acid has 15 times more affinity to bind calcium than that of calcium for oxalate.^[12] In our study 8 (26.67%) patients shows oxalate level above 40 mg/24 hrs, 12(40%) patients had oxalate level between 30-40mg/24 hrs and 10 (33.33%) patient shows oxalate level $<30\text{mg}/24\text{hrs}$. In our study oxalate excretion was in range of 29.9 ± 0.0 to 43.2 ± 4.9 with overall mean of 35.46 ± 8.96 . A study by *Singh et al.* noted that hyperoxaluria was seen in 43.2% cases.^[9] In another series *Singh et al.* noted that hyperoxaluria was seen in 45.1% cases. They also reported that dietary oxalate contributes as much as 50% of urinary oxalate and that this is single most important determinant of stone formation in many patients.^[13]

Sharda et al. found that the mean oxalate level in cases was 40.90 ± 1.957 (n=100).^[14] *Yukio et al.* shows that mean oxalate level in cases was 47 ± 23 . After comparing our results with other we can say that hyperoxaluria is important risk factor in stone formation in this region.^[14]

Phosphate is a benign component of urine but with persistent infection in alkaline urine it may gives rise to calcium phosphate stones. In our

study level of mean phosphorus in cases was 488.6 ± 132.8 .

Disturbance in uric acid metabolism is important not only for uric acid stones but also for calcium stone formation.^[15] We find that mean uric acid in cases was 419.4 ± 107.7 . *Sharda et al.* reported mean uric acid in cases was 485.9 ± 31.64 (n=100).^[10] *Gyawali PR.* shows that urinary uric acid in cases was 525.2 ± 261.2 .^[11]

In our study level of mean magnesium level in cases was 73.6 ± 5.7 . A study by *Sharda R. et al.* shows that mean magnesium level in cases was 113 ± 12.5 (n=100).^[10] *Jawalekar et al.* in her study also finds less calcium levels in urine of stone formers.^[6] The decreased magnesium in nephrolithiasis results in increased urinary oxalate level, as sufficient magnesium is not available to form the magnesium oxalate complex.

Urinary citrate is an important stone inhibitory substance for its ability to complex with calcium thereby withholding the formation of calcium oxalate.^[16] In our study level of mean citrate level in cases was 287.2 ± 36.8 and hypocitraturia was present in 73.33% cases, and this is comparable with the study of *Julka et al.* who find hypocitraturia in 77% cases.^[4] *Singh PP* shows that mean citrate level in cases was $301\text{mg}\pm 12\text{mg}$ and he find that 57.3% patients were hypocitraturic. *Rafique Anjum et al.* find hypocitraturia in 78% cases.^[17] In contrast to above studies *Sharda R. et al.* shows that mean citrate level in cases was 148.9 ± 5.035 (n=100) much below than our findings.^[14] In western world literature hypocitraturia seen in 30% cases.^[18] So after comparing with other studies we can say that hypocitraturia is an obvious finding in our study.

Tamm- Horsfall Protein was first described as a normal constituent of urine in 1950 and has been found to be the main constituent of hyaline cast and is filamentous in nature with an

ability to aggregate. Broadly on excretion of <60mg/day is suppose to exert inhibitory effect, where as a level of >60mg/day it acts as a promoter. THP is much stronger inhibitor than citrate, but on weight basis it exerts much less influence than citrate due to its molecular weight.^[19]

In the present series excretion of urinary THP was in the range of 32.9±0.0 to 42.07±3.73 in cases with the mean value of 37.2± 5.4. In controls the urinary THP was in the range of 36.6±9.4 to 45.6±11, with the mean value of 44.4± 10.8. Accordingly we can say that THP did not behave as a risk factor in urinary calculogenesis.

Conclusion

Our observation although in a limited number of cases gives an incling that hypocitraturia present in 73.33% cases is by far the biggest risk factor followed by hypercalciuria, with mild

hypercalciuria present (calcium excretion between 200-250mg/day) in 43.33% cases, moderate hypercalciuria present (calcium excretion between 250-300mg/day) in 23.33% cases and severe hypercalciuria (calcium excretion >300mg/day) in 16.66% cases. The oxalate excretion varies in selected population and mild hyperoxaluria (oxalate excretion >40mg/day) present in 23.33% (n=7) cases and moderate hyperoxaluria (oxalate excretion >50mg/day) present in 3.33% (n=1) case only Normal urinary excretion of uric acid in all patients. Needless to say that that the selected healthy individuals (controls) shows excretion of promoters and inhibitors in urine within normal limits except mild hypercalciuria was present in 9(30%) cases and mild hypocitraturia present in 4(13.33%) cases. and THP level was on higher side in 2(6.66%) healthy individuals only.

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