Inhibition efficiency of urine towards stone forming minerals.

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Abstract :

Urine specimens were collected from 100 normal individuals and 100 kidney stone patients and analysed spectrophotometrically for common stone promoters like oxalate, calcium, uric acid and phosphate and stone inhibitors like citrate and magnesium. Inhibition efficiency by these inhibitors exiting in urine samples towards the mineralisation of urinary stone forming minerals like calcium oxalate ,phosphate or carbonate has been studied in an experimental model. The results were presented as mean ± SD, student's t test was used for statistical analysis. Hypocitraturia and hyperoxaluria were the common abnormalities in the stone formers. Kidney stone patients had significantly higher urinary oxalate, calcium and uric acid and lower phosphate than normal individuals. The efficiency is markedly higher for phosphate, carbonate and urates by healthy urine samples.For the stubborn mineral oxalate has low inhibition efficiency. The urine of stone formers show very little inhibition. This implies that inhibition of crystal growth is proportional to the concentration of inhibitory factor present.

Keywords :- kidney stone, promoters, inhibitors, hypocitraturia, hyperoxaluria, Crystallization.

Introduction

The most important etiological factor in the formation of urinary stones is saturation of urine, and thermodynamic solubility product (Ksp) is the concentration at which saturation is reached and the process of crystallization in a solution initiated. Urine is a complex solution containing ions constantly interacting with calcium and phosphate. Formation product (Kf) of a particular salt is the concentration at which urine can hold no more salt in solution, at a particular temperature and PH.[1]

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. 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. [2]

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.[3] The mechanism of inhibitory action of citric acid is probably through the chelating of Ca^{++} ions in urine and thus, preventing the latter from combining with stone forming anions like oxalate, phosphate etc. To precipitate out.In –vitro studies have shown the sequesteration and chelation of urinary stone forming minerals by citric and other related natural acids.Inhibition of nucleation and aggregation of calcium oxalate by whole has been observed in in-vitro studies. [4] [5]

Magnesium a divalent cation is a complexing agent for oxalate. Magnesium inhibits oxalate absorption and excretion thus prevents its supersaturation. Normally magnesium is complexed with calcium as well as oxalate and decreases its excretion.[6]

Materials and Methods

The study included 100 patients with stone disease age ranges from 16- 60 yrs. Kidney stone patients were selected among those attending the local clinics . 100 healthy persons age ranges from 15- 58 yrs 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 based on plain abdominal X – ray, ultrasonography or intravenous pyelography. Patient who had history of bowel disease, renal tubular acidosis and urinary tract anomalies were excluded from the study. There were no dietary restrictions perse.

Inclusive criteria decided for the patients, who is only suffering with urolithiasis and the patients with urolithiasis and along with other disease are excluded from the study.

All subjects (healthy persons and stone patients) were in the dietary habit of mixed (vegetarian and non vegetarian) food. 24 hours urine out put was collected in presence of 1 % thymol, as a preservative. The samples were analysed after collection for uric acid, phosphates were investigated using kits from Erba diagnostics Ltd. Citrate, magnesium and calcium were estimated using standard procedure. [7] [8] [9] Oxalate was determined by titration with potassium permanganate. [10]

Estimation of urinary inhibition efficiency towards mineralisation

The procedure was followed as per T.V.R.K.Rao [11]

Crystalloid forming solutions namely solution of calcium acetate, trisodium phosphate, disodium oxalate and sodium carbonate of 0.01 M concentration and NaCl and uric acid were prepared in double distilled water. All chemicals were of analytical reagent grade. An experimental model was designed in which the two salt forming solutions, that is sodium phosphate and calcium acetate (for calcium Phosphate) disodium oxalate and calcium acetate (for Calcium oxalate) sodium carbonate and calcium acetate (for calcium carbonate) sodium chloride and uric acid (for Sodium Urate) were taken in two separate burettes (50 ml) and were allowed to fall simultaneously and slowly (drop wise) at the rate of 25 ml / hour with into a 250 ml beaker containing 50 ml of urine sample (it contains natural metabolic inhibitors like citrate and magnesium). The whole operation took about 2 hours. At the end of titration the contents of beaker were digested in a boiling water bath for 15 minutes, cooled to room temperature.Total precipitate was collected by centrifugation at 4000rpm for 15 mins. into a pre-weighed centrifuge. The tube with the precipitate was dried in hot air oven at 120^0 C, cooled to room temperature and weighed till constant weight. Weight of the precipitate was determined.

Simultaneous blank experiments with water in place of urine sample were also carried out for evaluating the inhibition efficiency of urine compared to that of water. All experiments were carried out at room temperature.

Percentage efficiency of inhibition by the urine sample was calculated using the formula,

$$\text{Percentage} = \frac{\text{weight of ppt. in blank set} - \text{weight of ppt. in exptal. Set}}{\text{Inhibition}} \times 100$$

Weight of ppt in blank set

Blank Set = Water as inhibitor that i.e.zero concentration of inhibitors

Exptal Set = Urine samples as inhibitor.

Result

Table: 1 showing Laboratory findings of 24 hours Urinary Constituents

Parameters	Controls	Stone formers	P Value
Oxalate (mmol/24 hrs)	0.29 ± 0.07	0.45 ± 0.19	0.0000
Citrate (mmol/24 hrs)	1.94 ± 0.29	1.00 ± 0.35	0.0000
Calcium (mmol/24 hrs)	4.00 ± 0.96	11.59 ± 8.00	0.0001
Uric acid (mmol/24 hrs)	2.58 ± 0.64	3.47 ± 1.31	0.01
Phosphate (mmol/24 hrs)	31.80 ± 6.06	32.21 ± 8.79	0.813
Magnesium (mmol/24 hrs)	3.82 ± 1.72	2.38 ± 1.23	0.01

NOTE:- Values expressed mean ± Std Deviation, NS:- Not Significant

The Table 1 Show the common promoters of stone formation in urine like calcium, oxalate, phosphate, uric acid and common inhibitors like citrate and magnesium compared between stone formers and controls. The urinary oxalate, calcium and uric acid concentration were persistently higher in stone patient when compared with normal individual ($P < 0.00$, $P < 0.0001$, and $P < 0.01$ respectively), but no difference was found in phosphate excretion. Urinary citrate and magnesium levels were low in stone patients as compared to normal individuals.

Hypocitraturia was the most common abnormality found in stone formers. Hyperoxaluria was the second most common abnormality.

Table: 2 showing the level of stone forming minerals among cases and control

Inhibitory efficiency in % towards the mineral

Parameters	Controls	Stone formers	P Value
Calcium Phosphates	35.76 ± 12.81	14.54 ± 4.99	0.0005
Calcium oxalate	22.56 ± 7.30	7.42 ± 2.34	0.0005
Calcium Carbonate	47.30 ± 8.61	16.97 ± 5.27	0.0005
Uric acid	66.16 ± 11.11	33.43 ± 7.49	0.0005

NOTE:- Values expressed mean ± Std Deviation, NS:- Not Significant

Table 2 shows the inhibition efficiency of urine samples towards stone forming minerals. The efficiency however, is markedly higher for phosphate and carbonate and urate. The inhibition efficiency varies in the range 23-45 % Mean (35.76 ± 12.81) for phosphate and 39 – 55% Mean (47.30 ± 8.61) for carbonate, for uric acid 55 – 77 % Mean (66.16 ± 11.11) by the healthy urine samples. For the stubborn mineral, oxalate, the urine generally has low inhibition efficiency 15 –29% Mean (22.56 ± 7.30) by healthy urine samples.

The urine of stone formers shows very little inhibition. The observed inhibition efficiencies are 10 - 18 % Mean (14.54 ± 4.94) for phosphate, 11 – 21% Mean (16.97 ± 5.27) for carbonate, 26 –40 % Mean (33.43 ± 7.49) for urate and 5 – 9 % Mean, (7.42 ± 2.34) for oxalate. This may presumably, be due to very low citrate and magnesium values at the same time increase values of calcium,oxalate urates in these urine samples.

Discussion

Normally in urine, the concentration of calcium oxalate is four times more than its solubility, and precipitation occurs only when supersaturation is 7 –11 times its solubility.[12] Individuals who have never formed stone often pass small crystals, which is possible because of presence of calcium oxalate crystallization modifiers in the urine. Calcium stone formers excrete significantly more calcium and oxalate than normal subjects and non-calcium stone formers. But not all individuals who excrete more calcium and oxalate in urine form calcium stones, which is due to presence of inhibitors in the urine. Stone formation is dependent on the balance between concentrations of promoters and concentration of inhibitors in urine.

Organic and inorganic inhibitors have been identified for calcium –phosphate and calcium oxalate systems, but not for the urate system which depends on acidity of urine.

Complexing agents are substances that form complexes with lattice ions for specific crystals (example: calcium Oxalate) and these agents decrease free ionic activity reducing the saturation level of the stone forming substances. Citrate is a potent complexing agent for calcium and magnesium a divalent cation, is a complexing agent for oxalate.

A study of the inhibition efficiency of urine samples towards mineralisation of stone forming minerals indicates that the human urine has some efficiency in itself to inhibit the mineralisation .[11] The data of stone formers shows that most of the patients present with low urinary citrate level and at the same time increased calcium level.This results in a loss of balance between citrate (inhibitor) and calcium (promoter). Citrate's capacity to sequester ate calcium ions is lost. The stone forming anions of urine like oxalate, phosphate etc. now gets an upper hand to react with Ca^{++} and thus precipitate it out. Urinary citrate and calcium values in study of inhibition efficiencies suggests that the two factors are directly proportional to each other. 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.

The factors serve as an ideal mechanism for the crystallization of uric acid in the urine are are 1) Excessive excretion of acid urine at relatively fixed low urinary PH. 2) Absorb, produce and excrete more uric acid than patients without gout to uric acid stone. 3) Diminished urine volumes. Uric acid further promotes calcium oxalate crystallization by facilitating the formation of nuclei. Addition of crystal of uric acid to the supersaturated calcium oxalate solution induces the deposition of well-oriented crystal of calcium oxalate over the uric acid. Sodium acid urate also nullifies the effectiveness of naturally occurring inhibitors of calcium oxalate crystal growth. [13]

The decreased magnesium in nephrolithiasis results in increased urinary oxalate level, as sufficient magnesium is not available to form the magnesium oxalate complex. Low urinary magnesium from an inadequate intake may accentuate urinary saturation of calcium oxalate because of inadequate complexation of oxalate by magnesium. [6] These observations indicate that the function of magnesium in stone formation is direct inhibition of calcium oxalate crystallization in the urine, causing an increase in urinary citrate excretion.

This study shows higher degree of crystal growth in patients in the presence of lower concentration of inhibitors as compared to normal individual . This implies that inhibition of crystal growth is proportional to the concentration of inhibitory factor present, which is invariably the case indirect study of inhibition in vitro. To date, the inhibition effect has been generally ascribed to a variety of urine inhibitory factors including citrate, Magnesium.

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