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ORIGINAL ARTICLE

Evaluation Of Urinary Abnormalities In Urolithiasis Patients From "Kathmandu Region"

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ABSTRACT

We evaluated urinary abnormalities in 193 consecutive patients with renal stones, without any prior history, and compared them with age /sex matched healthy controls. Over a twenty four hour period, urinary oxalate, calcium, uric acid, struvite, cystine, sodium, magnesium, phosphorous and citrate were estimated. The urinary pH was also determined. In stone formers, the twenty four hour period evaluation levels of calcium (CI; 183.4 – 185.2), oxalate (CI; 43.4 - 43.8), uric acid (CI; 518.3-528.5) and sodium (CI; 230.9-232.1) excretion were significantly higher ($p<0.001$), as compared with controls. The twenty-hour urinary citrate (CI; 151.8- 153.1), phosphate (CI: 241.7-245.7) and struvite (CI; 362.8 -372.1) excretion was significantly lower than the control group ($p<0.001$). The cystine (CI; 454.3- 459.5) excretion in stone formers was significantly higher ($p<0.05$) as compared to the control group. The magnesium (CI; 123.1- 124.3) excretion was lower in stone formers as compared to controls, and was significant ($p<0.01$). The pH of urinary stone formers was 5.13 ± 0.034, whereas in controls, it was 6.54 ± 0.045 ($p< 0.001$). Hypocitraturia is the main cause of renal calculi, along with hypomagnesuria and hypophosphaturia in the patients of Kathmandu region. On the basis of urinary abnormalities, further stone formation in the patient can be prevented by dietary modifications.

Key Words: Hypocitraturia, Hypomagnesiuria, Urolithaisis, Renal calculi, Nepal.

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Introduction

Renal lithiasis can be defined as the consequence of an alteration of the normal crystallization conditions of urine in the urinary tract. In a healthy individual, during the residence time of urine in the urinary tract, crystals either do not form, or are so small that they can be eliminated uneventfully (asymptomatic crystalluria). When normal urine crystallization conditions become altered, the rate of crystal nucleation and growth may become such that the crystals cannot be easily eliminated due to their size results in urolithiasis. In some cases, altered urinary conditions affecting crystallization are related to specific underlying disorders such

as hyperparathyroidism, which is associated with hypercalciuria[1] tubular acidosis, which is associated with hypercalciuria and hypocitraturia [2], and some genetic alterations which are associated with Hyperoxaluria[3] hypercystinuria[4] and hypercalciuria [5] However, in many cases, it is not possible to clearly identify the underlying disorder. Indeed, in nearly all renal calculi cases, crystal formation is attributable to a combination of diverse factors that may or may not be associated with an underlying disorder. Urine composition factors are important in crystal formation, as urine is a metastable liquid containing several coexisting substances that can crystallize to generate renal calculi. These substances are present at supersaturated levels, meaning that the urine is in an unstable state, and a stable urine state will eventuate through crystallization of the excess solute. The ease of crystallization depends on the degree of supersaturation, the presence of preformed particles (so-called heterogeneous nucleants that act as promoter substances), and the level of crystallization inhibitors. These latter substances inhibit crystal nucleation and/or growth. In the era of advanced ultrasound scanning, most renal stones are diagnosed without any symptom. It strikes without warning at any time, and the pain is often described as worse than labour pain. The diverse manifestations of urolithiasis provide a very interesting epidemiological study from the standpoints of geography, socioeconomic status, nutrition and culture. Stone disease not only affects the patients, but also the national economy, as the disease in prevalent in the productive age group[6]. There has been a continuous search for the cost-effectiveness of different treatment modalities, not only to treat the patients, but also to prevent its recurrence. This is one of the major public health problems in the developing and under developed countries, where the problem of endemic bladder stone is common due to dietary habits mostly derived from cereals grains, rather then meat[7]. Alkaline beverages are highly effective in preventing the recurrence of calcium oxalate, uric acid and cysteine lithiasis8. Epidemiological and clinical based studies have focused on the dietary aspects for the prevention of renal stones9. Factors that precipitate the formation of urinary stones are climate, diet, genetics, state of hydration and bacterial colonization.[11]

Considering the variation in causes of kidney stone, the current study was undertaken to find out the exact cause of renal calculi formation in the Kathmandu region, the capital city of Nepal.

Patients And Methods

Selection Of The Patients

One hundred and ninety three patients (116 males and 77 females; male: female 1.5:1) suffering from Urolithiasis between the age group of 26 to 54 years who had undergone surgery at the Department of Surgery, Nepal Medical College, constituted the test group. The diagnosis of Urolithiasis was confirmed by imaging test i.e. ultrasonography. Patients suffering from Urolithiasis for the first time were included in the test group.

Exclusion Criteria: Patients suffering from any other diseases were excluded from the study.

Dietary Habits

All the patients consumed a diet which consisted of tomatoes, spinach, beans, cereals, groundnuts, soybean seeds, and frequently, a non- vegetarian diet which consisted of beef and goat, accompanied by regular consumption of local hand made brew C*hang and Rakshi,* and widespread consumption of tea and coffee. The source of drinking water was mainly from *Dhunge Dhara* (natural water from mountains) and from the Nepal Govt drinking water supply.

Control Group

One hundred and ninety three normal healthy individuals selected from Kathmandu region (116 males and 77 females; male: female 1.5:1) between the

age group of 26 to 54 years, constituted the control group. All control groups were free from diseases including urolithiasis.

Collection Of Samples

The twenty-four hour urine samples were collected in a wide capped clean transparent graduated plastic container bottle. Preservatives such as 10N HCl were used for calcium and oxalate, 10N H_2SO_4 was used for citrate, and thymol crystals were used for sodium, uric acid, magnesium, phosphate and struvite. The samples were preserved in a refrigerator. The volume of the specimen was noted, and was used for the analysis of biochemical parameters. For pH measurements, early morning samples were used.

The biochemical parameters such as Oxalate, Citrate, Uric acid, Calcium, Phosphate, Sodium and Magnesium, were analyzed using Sigma Diagnostics assay kits. The Human diagnostic chemistry analyzer was used to carry out all analyses.
Citrate was estimated using a colorimetric standard method[10]. Sodium and potassium levels were estimated by a Medica Easylyte Analyzer.

The pH of the urine was measured by using an ion selective electrode (Systronic µ pH system 361). The observations of each parameter were compared between those of controls and stone formers. The results were expressed as mean ± standard deviation (SD), and statistical analyses of the data were performed by student's unpaired t-test.

RESULTS

The twenty-four hour calcium, oxalate, uric acid and sodium excretion levels were significantly higher ($p<0.0001$) in stone formers when compared to normal healthy individuals. The twenty-hour urinary citrate, phosphate and struvite excretion levels were significantly lower than the control group ($p<0.0001$). The cystine excretion in stone formers was significantly higher ($p<0.05$), as compared to the control group. The magnesium excretion was lower in stone formers compared to controls, and was significant ($p<0.01$).
The pH of urinary stone formers was 5.13 ± 0.034, whereas in controls, it was 6.54 ± 0.045($p<0.001$).

[Table / Fig 1] Twenty four hour urinary constituents in first time stone formers and control group

Parameters	Stone formers *(n=193) (mean ± SD)*	Control *(n=193) (mean ± SD)*	P value (95% CI)
Calcium (mg/24 hrs)	184.3 ± 6.7	90.8 ±4.3	<0.001 (183.4-185.2)
Oxalate (mg/24 hrs)	43.6 ± 1.7	23.4 ±1.2	<0.001(43.4-43.8)
Uric acid (mg/24 hrs)	523.4 ± 36.2	289.5 ± 9.8	<0.001(518.3-528.5)
Cystine (mg/24 hrs)	456.9 ± 18.4	325.3 ± 13.6	<0.05(454.3- 459.5)
Struvite (mg/24 hrs)	367.4 ± 32.4	564.7 ± 12.4	<0.001(362.8 -372.1)
Magnesium (mg/24 hrs)	123.7 ± 4.6	153.6 ± 13.3	<0.01(123.1- 124.3)
Phosphate (mg/24 hrs)	243.7 ± 14.3	453.3 ± 7.8	<0.001 (241.7-245.7)
Sodium (mEq/24 hrs)	231.5 ± 4.5	174.6 ± 3.5	<0.001 (230.9-232.1)
Citrate (mg/24 hrs)	152.7 ± 4.8	411.2 ± 6.3	<0.001(151.8- 153.1)

Statistical analysis was performed by student's t-test

Discussion

Urinary stone disease is a major public health problem in many parts of the world, and is endemic in India and Nepal11. In the current study, twenty-four hour urinary calcium, oxalate, uric acid, cystine and sodium concentrations were higher in stone formers. These are the major risk factors in first time stone formers and in recurrent stone formers.[12].
The current study assumes that increased levels of calcium in tubular filtrates might be due to abnormalities in calcium metabolism. It might be at the hormonal level or at the level of ossification of bone (dysfunction of osteoclasts and osteoblasts), which needs to be further established.

It could also be idiopathic calculi formation, where the main epidemiological factors are age, sex, gender, season, climate, stress, occupation, affluence, diet (including fluid intake), and genetic and metabolic factors. The role of diet has been extensively studied, and the changing pattern of diet determines the type of stone formation. Rich animal protein, refined sugar and salt, also increase the incidence of renal stone. Formation of calcium calculi implies the existence of an altered (damaged or partially injured) papillary epithelium. This can be a consequence of cytotoxic substances that also can induce sub-epithelial calcifications. Moreover, renal calculi of this type are frequently associated with a deficiency in the levels of crystallization inhibitors and Hyperoxaluria.

Uric acid induces the heterogeneous formation nucleation of calcium oxalate stone. It was first proposed that uric acid crystals adsorb and inactivate natural urine inhibitors of stone like glycosaminoglycans[13]. The higher concentration of urate in urine may be due to high intake of a purine rich diet, and hence, increased concentration of urate causes precipitation of calcium oxalate crystals. The higher incidence of uric acid lithiasis could also be due to excessive consumption of beef and alcohol consumption as a part of their dietary life style in stone formers, which was substantiated by the study conducted elsewhere[14], where they reported alcohol consumption and increased animal protein consumption as a major risk factor of stone formation. Urinary pH is an important variable that can be strongly affected by diet. A diet rich in animal protein is associated with high uric acid urinary excretion and a low urinary pH. Uric acid solubility decreases dramatically at a urinary pH lower that 5.5, leading to uric acid crystal formation. For people eating a vegetarian diet, the consumption of citrate-rich products (foods and soft-drinks) and carbonated beverages notably increases the urinary pH. Thus, the recommendations for uric acid stones involve liquid ingestion to produce daily urine volumes above 2 L, urine alkalinization using citrate or bicarbonate to maintain pH values between 6.2 and 6.5, and a predominantly vegetarian diet. Maintaining a moderate consumption of animal protein, seafood, and alcohol is also important. Treatment of this disorder requires periodic control of urinary pH to avoid excess alkalinization, which could cause other problems such as hydroxyapatite lithiasis.

Oxalate in urine may arise either as an end product of intermediary metabolism or from dietary sources[15]. Oxalate complexes with calcium to form calcium oxalate salt. The solubility of calcium oxalate stone is affected by the changes in the urinary pH. In the current study, the urinary pH of stone formers was 5.1 ± 0.03, which indicates that at this pH, calcium oxalate solubility is minimum and hence urine gets supersaturated with calcium oxalate. Hence, pH 5.1 ± 0.03 is the optimum pH for stone formation. This pH was favourable for stone formation in the control group. Even when stone was not formed, it might be due to the high levels of urinary inhibitors such as citrate, magnesium and phosphate.

The adequate dietary calcium, low animal protein and low salt decreases urinary excretion of both calcium and oxalate[16]. The excretion of sodium was significantly increased in stone formers, which might be due to high dietary intake of salt, which causes higher excretion of calcium and oxalate. Oxalate-rich foods may be yet another risk factor for formation of calcium oxalate monohydrate papillary calculi. A dietary oxalate excess is related to formation of calcium oxalate monohydrate calculi, and can mainly be related to an excess consumption of soybean seeds, nuts, spinach, chocolate and green tea. Studies have reported[17] that increased consumption of radish in the form of salad could be beneficial for calcium oxalate stone formers, as it increases the urinary excretion of calcium oxalate crystals.

In the current study, we observed a higher level of cystine calculi in stone formers. This is the ever first observation of cystine calculi in Nepal, as no such stone observation is cited in this country. A study conducted in Thailand[18] has reported five cases of renal stones at Ramathibodi hospital, the first case reported in Thailand. The main cause of cystine calculi is hypercystinuria, an autosomal recessive genetic disorder that causes increased renal cystine excretion. The hypercystinuria produces recurrent urolithiasis due to the low solubility of cystine at low urinary pH values, as observed in stone formers in the current study. The prophylactic measures are based on a high hydric ingestion (at least 4 L of water daily) and urine alkalinization using potassium citrate. When these measures are not sufficient, it is possible to use

complementary pharmacological treatment. A low methionine diet has been proposed to treat hypercystinuria. Methionine is an essential amino acid precursor of cysteine and cystine found in protein from both animal (meat, fish and eggs) and vegetable (soya, wheat and coconuts) sources.

In the current study, twenty-four hours urinary citrate, magnesium and phosphate excretion were significantly reduced in stone formers. These substances are known to be major inhibitors of crystallization of calcium oxalate and growth of the crystals.
The current study observed increased excretion of struvite infectious calculi in controls, as compared to stone formers. Usually the formation of struvite infectious calculi is due to urinary tract infection, and as treatment involves pharmacological (antibiotic) intervention to prevent recurrent infections, it is recommended that urinary pH should be maintained below 6.0 [19].

The intratubular citrate protonation depends on ambient pH. The low luminal fluid pH decreases free citrate and tolerable oxalate. The tolerable oxalate is the metastable limit of calcium oxalate solubility. The low luminal fluid pH drives the reabsorption of protonated citrate. Therefore, free citrate and tolerable oxalate are necessarily low. The lower citrate concentration may be due to distal tubular dysfunction. Magnesium inhibits oxalate absorption and excretion, thus preventing its supersaturation. Normally, magnesium is complexed with calcium as well as oxalate, and decreases its excretion. Thus, decreased magnesium concentration in urolithiasis results in increased urinary oxalate level, as sufficient magnesium is not available to form the magnesium-oxalate complex. The previous studies showed that urinary magnesium excretion was higher in children than adults, and hence, incidence of urolithiasis in children is very low as compared to adults in developed countries [20] Citrate-rich foods or citrate drugs are the basis of the most effective dietary or pharmacological treatment for calcium oxalate calculi due to the double effect of citrate acting as a crystallization inhibitor and increasing the urinary pH. Citrate and phytate are the only crystallization inhibitors that can be taken via the diet, or as a pharmaceutical. Citrate decreases calcium salt supersaturation due to its capacity to complex calcium ions, and also has crystallization inhibitor activity. Phytate, mainly present in whole cereals and legumes, can inhibit calcium salt crystallization, and individuals prone to calcium oxalate stone formation were found to have lower urinary phytate excretion, as compared to healthy subjects.

Dietary components influence the biochemical parameters such as oxalate, uric acid, calcium, and sodium etc. As in the Kathmandu region, the diet consists groundnuts, tomatoes, spinach, and animal proteins etc, use of more salt in dietary preparations may act as risk factors in addition to other risk factors for stone formation.

Preventive measures for avoiding each type of renal calculus formation, involve specific dietary considerations. While there are specific dietary factors to be considered for each calculus type, there is also a general list of dietary measures that can be recommended in order to avoid any renal calculus formation. The measurement includes daily intake of a suitable liquid volume (minimum 2 L water/day), a strict vegetarian diet, and excessive animal protein diets must be avoided. Excessive salt (NaCl), Vitamin C and/ or Vitamin D consumption should be avoided. It is advised to consume phytate-rich products (natural dietary bran, legumes and beans, whole cereals) and preferably avoid exposition to cytotoxic substances (i.e., analgesics abuse, residual pesticides, organic solvents and cytotoxic drugs). Biochemical analysis calls for the 24 hours urine sample analysis. For stones to go undetected in routine urine examination, the method described elsewhere should be adopted[21], as it increases the chances of detection of calculi.

The data presented in this study clearly demonstrate that renal stone is one of the major public health problems in Nepal, and it is associated with significant pain and suffering, as well as economic costs. Early detection and management could cover way for preventive steps that could be adopted to minimize the risk of renal stone disease.

Conclusion

Multiple aetiological factors are responsible for stone formation,.In the present study, it is concluded that hypocitraturia is the main cause of renal calculi, along with hypomagnesuria and hypophosphaturia in the patients of Kathmandu region.

On the basis of urinary abnormalities, dietary modifications such as low intake of groundnuts, tomatoes, spinach, animal proteins, salt etc can be suggested to the stone formers.

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