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Variability of protein content and its role in kidney stone formation

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ABSTRACT

The current study was carried out to extract the clinical history as well as data from the affected patients suffering from chronic renal failure due to the presence of Kidney Stones. Further, the study was directed to find out the root cause and types of kidney stones and symptoms involved in the same. The protein compositions of the stones were also studied by collecting different types of kidney stones to correlate the patient's clinical data by comparative study and finally this study indicates that the diet is the main causative factor for kidney stone formation. It can only control through a proper diet and risk factor is irrespective of age, sex, blood group.

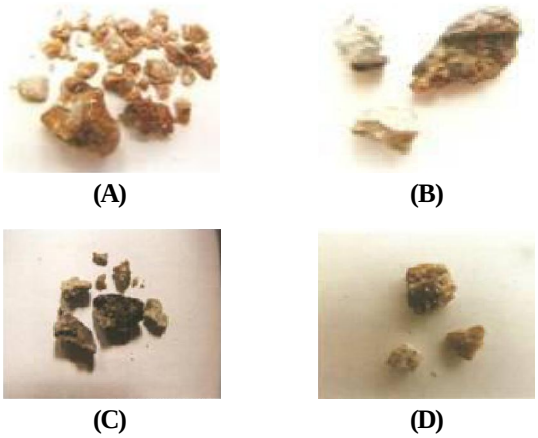
KEYWORDS

Kidney stone;
SDS-PAGE;
Variability of protein;
Comparative study.

INTRODUCTION

Kidney stones, or Renal calculi, are solid concretions (crystal aggregations) of dissolved minerals in urine; calculi typically form inside the kidneys or ureters[1]. The terms nephrolithiasis and urolithiasis refer to the presence of calculi in the kidneys and urinary tract, respectively. Renal calculi can vary in size from as small as grains of sand to as large as grapefruit. Kidney stones typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size before passage on the order of at least 2-3 millimeters, they can cause obstruction of the ureter[2]. The resulting distention with urine can cause severe episodic pain, most commonly felt in the flank, lower abdomen and groin (a condition called renal colic). Renal colic can be associated with nausea and vomiting due to the embryological association of the kidneys and the intestinal tract. Recurrence rates are estimated at about 10% per year[12].

Kidney stones are most commonly composed of calcium oxalate crystals, and factors that promote the precipitation of crystals in the urine are associated with the development of renal calculi. Conventional wisdom and common sense has long held that consumption of too much calcium can promote the development of kidney stones[3]. However, current evidence suggests that the consumption of low-calcium diets is actually associated with a higher overall risk for the development of kidney stones. This is perhaps related to the role of calcium in binding ingested oxalate in the gastrointesti-



(A) (B)

(C) (D)

Figure 1 : Types of kidney stones: (A) Calcium oxalate stones, (B) Calcium phosphate stone, (C) Uric acid stone, (D) Struvite stone

nal tract. As the amount of calcium intake decreases, the amount of oxalate available for absorption into the bloodstream increases; this oxalate is then excreted in greater amounts into the urine by the kidneys. In the urine, oxalate is a very strong promoter of calcium oxalate precipitation, about 15 times stronger than calcium[4].

Other components of kidney stones include struvite (magnesium, ammonium and phosphate), uric acid, calcium phosphate, or cystine (found only in high urinary concentrations in people suffering from cystinuria). The formation of struvite stones is associated with the presence of urea-splitting bacteria, most commonly *Proteus mirabilis* (but also Klebsiella, Errata, Providencia species). These organisms are capable of splitting urea into ammonia, decreasing the acidity of the urine and resulting in favorable conditions for the formation of struvite stones[10,11]. The formation of calcium phosphate stones is associated with conditions such as hyperparathyroidism and renal tubular acidosis. The formation of uric acid stones is associated with conditions that cause high blood uric acid levels, such as gout, leukemias/lymphomas treated by chemotherapy (secondary gout from the death of leukemic cells), and acid/base metabolism disorders[3]. Renal calculi can occur due to other underlying conditions, such as renal tubular acidosis, Dent's disease and medullary sponge kidney, Many centers will screen for such disorders in patients with recurrent nephrolithiasis[5].

SDS containing Polyacrylamide gel separates the protein molecules in the order of their molecular mass because of gel filtration effects. PAGE consists of a two gel system caste between two glass plates and several different buffers. The running gel or resolving gel in which the separation takes place, which is over layered by a short (1cm) large pored stacking or spacer gel. The gel running unit consists of two reservoir in which buffer is filled .The upper reservoir consisting positive electrode and the lower reservoir consisting of both reservoirs will be filled with the running buffer while conducting the electrophoresis.

MATERIALS AND METHOD

Sample collection

Kidney stones

The clinical samples and data of the patients i.e., age of the patient, blood group and sex were randomly collected from the nearby hospitals like J.S.S .Hospital and Hi-Tech Kidney Stone center, Mysore, Karnataka state, India (TABLE 1). Patients were selected on a random basis irrespective of their age and sex and blood group. The collected kidney stone samples were washed with distilled water and stored in saline at 4ºC until further analysis[6].

Isolation of proteins from kidney stones

(a) Processing of kidney stones

Kidney stones were taken on a clean paper towel, the extra water was removed by pressing between paper towel. One gram of each sample were weighed accurately and transferred into a clean sterile mortar. These stones were thoroughly grinded with 2ml of phosphate buffer saline (PBS) (pH 6.8) using a pestle and mortar to obtain a fine suspension. The suspension was centrifuged at 10,000 rpm for 15 min at 4°C using an cooling centrifuge (eppendorf 5810, Germany). The supernatant was transferred into clean eppendorf tubes and stored at 4°C for further use.

(b) Protein estimation

The protein concentrations in the supernatant extracted from stone were estimated using Bradford's method [Bradford, 1976], 50µL of the sample taken in a clean cuvette and O.D was taken at 595nm, a standard graph was plotted to get protein concentration.

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TABLE 1 : Patients details used in the present study

Si No.	Patient	Age	Sex	Blood group
1	Patient Number 1	42	F	O+
2	Patient Number 2	63	F	A+
3	Patient Number 3	40	M	O+
4	Patient Number 4	42	M	O+
5	Patient Number 5	46	M	B+
6	Patient Number 6	38	F	A+
7	Patient Number 7	55	M	O+
8	Patient Number 8	45	F	A+
9	Patient Number 9	19	M	A+
10	Patient Number10	50	M	O+

Protein profiling by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS containing Polyacrylamide gel separates the protein molecules in the order of their molecular mass because of gel filtration effects. 12.5% SDS-PAGE was prepared by using a standard protocol (Molecular cloning, a laboratory manual, 2nd edition, Sambrook and Russel) and 100µL of the sample (18 µg /well concentration) and equal amount of sample buffer was added, protein denaturation was done on a boiling water bath for 10 minutes. The samples were loaded into the stacking gel wells and it was run at 40mV, after the sample enters separating gel it was run at 80mV. Finally gel was stained using coomassie brilliant blue for 8h and destained using destaining solution[7,8].

RESULT

Kidney stones and patients data was collected from JSS Hospital and Hi-tech kidney stone centre showedvaried size, the size of each kidney stone varied from 1-3mm (Figure 1). Based on the morphological appearance kidney stones were categorized into calcium oxalate stones, calcium phosphate stones, uric acid stones and struvite stones.

Total protein contents of kidney stones

The amount of protein present in different samples recorded varied concentration ranged from 1.5-4.1 µg. The protein concentration from kidney stone from patients 9 and 10 yielded the highest amount of protein 4.1µg. This was followed by sample number 8 and 7 yielding 3.7 and 3.4 µg respectively. Sample number 5 and 6 yielded 3µg each of protein, rest of the samples expressed protein concentration ranging between 1.5 and 2.8µg. This varied concentration of protein in the samples clearly indicated the protein concentration defers irrespective of age, sex and blood group.

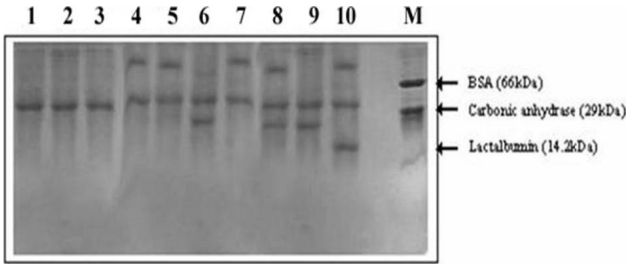

Figure 2 : Protein profiling by SDS-PAGE: The electrophorogram obtained from different Kidney stones. Concentration of protein loaded-18µg/well, the 12.5% SDS-PAGE was run at 40mV for stacking gel and 80mV for separating gel. The gel was stained in Coomassie blue for 8 hrs. Lane 1-10:sample of patients, Lane M: marker

Protein profiling by SDS-PAGE

The electrophorogram obtained after SDS PAGE exhibited differential banding pattern (Figure 2). Sample number 10 exhibited a unique band of 35.1kDa, apart from this it exhibited two more bands of 67KDa and 97 KDa. Samples number 6 and 8 exhibited similar banding pattern consisting of 95 KDa, 67 KDa and 47.2 KDa but the bands were more prominent in sample number 8. Sample number 1, 2 and 3 exhibited identical banding pattern consisting of two bands each of 140 and 60.4 KDa. Sample number 4, 5 and 7 exhibited similar banding pattern consisting of 2 bands each of 112KDa and 67KDa. It was interesting to note that the 67KDa proteins was present in almost all the samples such differential expression of banding pattern exhibited by different samples correlates well with the result of different scientific works carried out. The patients were categorized into different groups based on their sex, age and blood group.

DISCUSSION

The clinical samples and data of patients collected from the nearby hospitals like J.S.S. Hospital and Hi-Tech Kidney Stone Center, Mysore .Kidney stone disease does not show sex biases of the randomly selected 10 patients both men and women were in equal

strength.

According to the collected data of the patient people of blood group O+ and A+ were more prone to form kidney stone, i.e. among 10 patients examined 5 patients were O+ and other 4 patients belonged to A+ also it was found that the once who showed A+ were women while men showed O+ . This kidney stone formation does not depend on patient's age, sex and blood group. Although, from the present studies most of the patients who were in the age group of 40-50 showed more stone formation when compared to age group between 30-40 and 50-60 (TABLE 1).

CONCLUSION

It is difficult to accept recurrent stone formation as incidental in any patient and allow it to continue without efforts to understand its causes and offer such treatments as seem appropriate. Available trials offer physicians excellent treatment strategies for prevention of calcium stones, and since uric acid stones are a consequence of low urine pH, physicians can treat them confidently despite the lack of prospective trials for additional therapeutics[9]. Even cystine stones can be prevented, albeit with imperfect remedies. But treatments may pose their own problems. Although potassium citrate salts are effective, they, along with extracorporeal shock wave lithotripsy (ESWL), may promote the formation of calcium phosphate stones, the prevalence of which continues to rise with time, newly developed medical expulsive theory treatment comprises the use of drugs to help the spontaneous passage of ureteral calculi. Several drugs including calcium channel blockers (nifedipine), steroids, and α adrenergic blockers have recently been investigated. This is a significant area of interest that requires new research. Abnormal urine pH and calcium excretion rate are predominant findings in stone formations that play a major role in the pathogenesis of stone formation and our finding also show that the association between dietary factors and the kidney stone formation[8]. Kidney stone formation will be irrespective of age, sex and blood group .It can only controlled through a proper diet and more research is needed to identify the mechanism by which age, sex blood group affects the risk of stone formation.

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