Original Paper

Composition of Renal Stone– An experience at Armed Forces Institute of Pathology

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Abstract

Introduction: In Asia the stone belt has been reported to stretch across our neighbouring countries Pakistan, India, Myanmar etc signifies a higher incidence of renal stone disease in Bangladesh.

Objective: To determine the pattern of chemical composition of renal stones by semi-quantitative technique in patients presented to Armed Forces Institute of Pathology (AFIP) and to evaluate the predominant constituent present in them.

Materials and Methods: This descriptive study was conducted at Armed Forces Institute of Pathology (AFIP), Chemical pathology department from October 2013 to October 2014. Renal stones of 37 Urolithiasis patients were analyzed chemically, using DiaSys analysis kit, employing titrimetric method for estimation of calcium and colorimetric method for Oxalate, Ammonium, Phosphate, Magnesium, Uric Acid and Cystine. Concentration of each individual component then was expressed in percentage and used to interpret renal stone composition using the calculation scale.

Results: Males were more prone to renal stone disease, having male to female ratio 5.2:1. Urinary stones occur in all age groups, in this study age ranged from 4 to 72 years with mean age 38.8±16.0 years and mostly affected was the working age group 21 to 50 years (70.2%). Mixed components (i.e. mixed stone) rather than a single component was the commonest type constituting 83.8% of all renal stones. The commonest mixed stone found was Calcium Oxalate with Apatite (41.9 %). Pure Calcium Oxalate was the 2nd most common (10.8%) variant followed by Struvite stones (5.4%). Cystine and Brushite were the least common renal stones in this study.

Conclusion: Calcium Oxalate was the most predominant chemical component in renal stones (94.6%). More research is needed to assess the frequency, types, and correlation of renal stones with environmental, dietary and genetic factors in Bangladesh.

Key-words: Renal stone, Semi-quantitative dry chemical spot tests, Calcium Oxalate, Calcium phosphate, Urate, Struvite.

Introduction

The history of renal stones dates back to times of Egyptian mummies[1]. Urolithiasis is the third most common clinical problem of urinary system, occurring up to 15% of population in the western countries[2]. They are found in 1% of all autopsies[1]. In fact, nephrolithiasis is not uncommon, with a lifetime prevalence of 10% in men and 5% in women[3]. Besides, the recurrence rate for kidney stones is approximately 15% in 1st year and as high as 50% within 5 years of the initial stone occurance[4]. Most of the ureteral stones are less than 5mm and pass spontaneously[2].

In Asia the stone belt has been reported to stretch across Sudan, Saudi-Arabia, UAE, Pakistan, India, Myanmar, Thailand, Indonesia and Philippines[1]. In USA 2-3% of total population suffer from urinary stone diseases[5]. In Bangladesh, we have no statistics and it is more common in northern part of the country and predominantly affects male with a ratio of male to female is 3:1[5]. Kidney stones are most prevalent between the ages of 20 to 40 years[4]. As mostly the working age group is affected, makes it a major socio-economic burden on society.

There are different types of calculi. Most stones, 75-80% are calcium containing, composed largely of calcium oxalate followed by calcium phosphate. Another 10-15% is struvite composed of magnesium ammonium and phosphate, 6% are uric acid stones and 1-2% is cystine stones1. Nutritional, environmental and genetic factors are important lithogenic risk factors lead to urinary crystal growth, aggregation and deposition causing renal stone formation[6].

Knowledge of Chemical composition of renal stones can serve as a guide in the patient management through enhancing better understanding of the physico-chemical process in the formation of renal calculi. Low urine volume, or metabolic disorder results in super-saturation of urine with a particular element. When the concentration is high enough, it allows crystals to form or preformed crystals to grow or aggregate and ultimately form renal stone. Calcium oxalate or calcium phosphate stones are found in association with high concentrations of calcium, oxalate, uric acid or low citrate in urine; low urinary volume; high dietary sodium and protein intake[3]. Uric acid stones are related to acidic urine and hyperuricosuria. Struvite stones has link with urine infection mostly by urea-splitting bacteria. Cystine stones are allied with high urinary cystine concentration and acidic urine[3]. So, appropriate hydration and dietary restriction of sodium, animal protein, oxalate and uric acid rich food or intake of stone forming inhibitors like citrate, nephrocalcin, uropontin, and magnesium or alkalinization of urine with potassium alkali (e.g. potassium citrate) or sodium bicarbonate to dissolve uric acid and cystine stone or eradication of infection with appropriate antibiotics can contribute in both prevention and treatment of renal stone diseases[3]. Hence, determination of chemical composition of renal stone is an integral part for the proper management and prophylaxis of stone formation. This study was therefore conducted on chemical analysis of renal stone to know the composition of stone in patients reporting to AFIP for renal stone analysis.

Material and Methods

The study includes renal stones obtained from 37 patients, passed spontaneously then collected by patients themselves or removed through surgical intervention and presented to AFIP for chemical analysis during a period from October 2013 to October 2014.

The renal stones were analysed in the chemical pathology department of AFIP by Semi-quantitative dry chemical spot tests. At first the stones were washed with the distilled water to remove the debris, dried completely and weighed. Then the stones were crushed to powder with porcelain pestle and mortar. Approximately 15 mg powdered stone form was mixed with 5 drops of Sulfuric acid and then was mixed with distilled water in a graduated tube to make it up to 50 ml. This sample was then analysed by using DiaSys analysis kit This kit analyses Calcium by titrimetric method, basing on end point detection by counting the number of EDTA drops required for the colour change from red to blue in presence of indicator 'Calconcarboxilic acid'. The number of added drops is used for calculation of percentage of Calcium present in the renal stone. Oxalate, Ammonium, Phosphate, Magnesium, Uric Acid and Cystine are analysed by semi-quantitative colorimetric method through visual colour comparison using the colour scale contained in the kit. Concentration of each individual component was expressed in terms of percentage, which with the aid of 'calculation scale' was interpreted for renal stone composition.

Results

Total thirty seven renal stones were analysed by semi-quantitative chemical spot test. Among the subjects, highest age reported was 72 years and lowest was 04 years only. Mean age was 38.8±16.0 years. Majority of the cases (70.2%) reported were from working age group i.e. 21 to 50 years and highest incidence (40.5%) was observed among 41 to 50 years age group. The age distribution has been shown in the Table-I.

Table-I: Distribution of renal stone in different age groups (n=37)

Age in years	Frequency	Percentage
0 –10	03	8.1%
11 –20	02	5.4%
21 – 30	05	13.5%
31 – 40	06	16.2%
41– 50	15	40.5%
51 – 60	04	10.8%
> 60	02	5.4%

Out of 37 patients, 31 were male and 06 were female, with ratio 5.2:1. Most of the male patients (48.4%) suffered were from 5th decade, whereas most of the female patients (50%) belonged to 3rd decade.

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Table-II: Sex distribution of renal stone (n=37)

Sex	Frequency	Percentage	Male: Female
Male	31	83.8	5.2:1
Female	06	16.2	

Semi-quantitative chemical analysis revealed mixed stones were the commonest variant constituting 83.8% of all renal stones. Second most common type was pure calcium oxalate (10.8%) followed by struvite stones (5.4%). No pure Uric Acid or pure Cystine stone was found.

Table-III: Composition of Renal Stone (n=37)

Composition	Frequency	Percentage
Mixed stone	31	83.8%
Pure Calcium Oxalate	04	10.8%
Struvite	02	5.4%
Uric Acid	00	-
Cystine	00	-
Total	37	100%

Interestingly all the mixed stones (100%) had calcium oxalate in their composition. The commonest composition was calcium oxalate with calcium phosphate (45.1%) majority as apatite (41.9%) and remaining was brushite (3.2%). Calcium oxalate with Struvite (Mg-ammonium phosphate) was the 2nd common (19.3 %) variant. Calcium oxalate was present in 94.6% renal stones, whereas Cystine and brushite (Calcium Hydrogen Phosphate) were the least common variants. Frequency of compositions of mixed renal stones are shown in Table-IV.

Table-IV: Composition of Mixed Renal Stones (n=31)

Composition	Frequency	Percentage
CaOx + Calcium Phosphate (Apatite & Brushite)	14	45.1%
CaOx + Struvite (Mg-ammonium phosphate)	06	19.3%
CaOx + Struvite + Urate	02	6.4 %
CaOx + Ammonium Urate	02	6.4 %
CaOx + Apatite + Struvite	02	6.4 %
CaOx +Apatite + Ammonium Urate	02	6.4 %
CaOx + Urate	02	6.4 %
CaOx + Struvite + Cystine	01	3.2 %

*CaOx: Calcium oxalate; Apatite: Tri-Calcium phosphate; Brushite: Calcium hydrogen phosphate.

Discussion

As the stone belt in Asia stretches across our neighbouring countries like Pakistan, India, Myanmar etc indicates a higher incidence of renal stone disease in Bangladesh also. Chemical composition of renal stones presented to AFIP from October 2013 to October 2014 has been discussed in the study. The mean age calculated in this study subjects was 38.8 ± 16.0 SD years, with range 4 to 72 years. Shokouhi et al[7] found mean age 40.5 ± 15.1 years (2 - 86 years) is almost close to this study. In a study in Bangladesh by Hossain et al[5] reported mean age 41.5 years, is almost similar but a bit higher than this study because they excluded age group less than 18 years. Kashif et al[1] found mean age 43.9 ± 14.6 years, slightly higher than this, as age below 13 years were excluded in their study. The present study found mean age for female 24.3 years and for male 41.6 years, showing significant sex difference for mean age. Whereas, Shokouhi[7] showed a subtle insignificant sex difference for mean age, 42.2 years in males and 39.8 years in females. Though, mean age for male agree with this study but females had much lower mean age than their study may be due to less number of female study subjects in our study population. This study showed 70.2% cases were between 21 to 50 years, very similar to observation by Kashif[1], who found almost 69% of the patients were ranging between 20 to 50 years of age. So, mostly young working age groups are affected with renal tract calculi.

The male to female ratio in this study found is 5.2:1. In Riyadh[8] male to female ratio was 5:1, very similar to this, whereas, in Kathmandu[6] was 2:1, in Multan, Pakistan[9] was 3:1, in Ardabil, Iran[7] was 2.7:1, lower than the ratio observed in this study. All the studies revealed that men are affected more than female though the ratio was variable. Women typically excrete more citrate and less calcium than men which may partially explain the higher incidence of stone disease in men[4]. Besides, daily higher tissue breakdown owing to the larger muscle mass of men as compared to women results in increased metabolic waste is another predisposition of renal stone formation[1].

Composition of renal stone in this study revealed ‘mixed stones’ were the commonest variant (83.8%). ‘Pure calcium oxalate’ (10.8%) were the second common and this was followed by struvite stones (5.4%). No pure uric acid or cystine stone was found. The findings are similar to Gurau et al study[10] who also found majority of urinary stones have mixed composition (90.2%) and pure calcium oxalate (6.26%) was the second common variant. Chemical analysis of urinary stones in Larkana, Pakistan[2] also showed mixed stones (40.7%) are the predominant variety though the presenting percentage was much less than this and second common was pure calcium oxalate stones (33.1%). Interestingly study conducted at Department of Biochemistry, Nepal Medical College Teaching Hospital (NMCTH)[6] found 100% renal stones were mixed stones and there was no pure homogenous stone. Mixed stones composed of ‘calcium oxalate and calcium phosphate were the most prevalent type (45.1%) at AFIP, compatible with NMCTH[6] (65.9%). The second common mixed stones in this study of ‘calcium oxalate with struvite’ differs from NMCTH[6] of ‘calcium oxalate with calcium phosphate with uric acid’ stones (21.2%).

In this study, 10.8% renal stones were ‘pure calcium oxalate’ and 100% of ‘mixed stones’ had calcium oxalate in their composition and thus 94.6% of all renal stones had calcium oxalate in their composition. Different percentages of ‘pure calcium oxalate stone’ ranging from 26 to 63% have reported in different literatures[2,9,11-14]. The main etiologic factors[10] linked to such types of renal calculi are: low urine pH (<5.5), deficiency of crystallization inhibitors in urine (e.g. citrate, phytate), low urine volume, increased excretion of oxalate and calcium, high sodium intake, metabolic disorder like hyperoxaluria, exogenous or dietary intake of high oxalates: spinach, beets, walnuts, soy-based products, wheat bran, parsley, sesame, orange and lemon peel, milk chocolate, strawberries, tea, beer, vitamin-C supplementation etc.

In this study pure ‘struvite’ (magnesium-ammonium -phosphate) stone was the third common (5.4%) type. Besides, significant percentage (35.5%) of struvite was also present in mixed stones. All these mixed stones had calcium oxalate in common with additional presence of uric acid, calcium phosphate (apatite) or cystine in some cases. Struvite stones are often associated with infection[2]. Largely infections by bacteria e.g. Proteus, Providencia, Klebsiella, Pseudomonas, enterococi and some staphylococci convert urea to ammonia and CO_2 and raises the pH of urine.

Table-V: Comparison of chemical composition of renal calculi among different studies

Chemical composition of renal calculi	Present study AFIP, Bangladesh (n=37)	Kashif Bangash et al[1] PIMS Islamabad (n=232)	Rafique et al[9] Multan (n=700)	Javed Altaf et al[18] Jamshoro (n=100)	Ikram Ullah etal[19] Peshawar (n = 138)
CaOX + CaP	14 (37.8%)	37 (15.9%)	73 (10.4%)	5 (5 %)	16 (11.6%)
CaOx + Struvite ± Urate ± Cystine	09 (24.3 %)	Nil	Nil	Nil	Nil
CaOX	04 (10.8%)	89 (38.4%)	183 (26.1%)	30 (30%) (3% with aspartate)	53 (38.4%)
CaOx + UA	02 (5.4%)	74 (31.9%)	153 (21.8%)	35 (35 %)	29 (21%)
CaOX + Ammonium Urate	02 (5.4%)	7 (3%)	Nil	Nil	Nil
CaOx + CaP +Ammonium Urate	02 (5.4%)	6 (2.5%)	9 (1.2%)	Nil	Nil
CaOx + CaP + Mg /struvite	02(5.4%)	1 (0.43%)	Nil	Nil	Nil
Struvite (Mg-ammonium phosphate)	02 (5.4%)	4 (1.72%)	12 (1.7%)	5 (5%)	24 (17.4%)
Calcium Phosphate (CaP)	Nil	Nil	5 (0.7%)	7 (7%)	Nil
Uric Acid	Nil	1 (0.43%)	197 (28.1%)	10 (10%)	13 (9.4%)
CaOx + CaP + UA	Nil	11 (4.7%)	50 (7.1%)	8 (8%)	3 (2.2%)
CaP + UA	Nil	Nil	18 (2.5%)	Nil	Nil
CaOx + UA + Cystine	Nil	2 (0.86%)	Nil	Nil	Nil
Ammonium Urate	Nil	Nil	Nil	Nil	Nil

The resultant alkaline urine causes the precipitation of magnesium-ammonium- phosphate salts resulting in struvite stone formation[10,15]. No pure uric acid stone was noted in this study likewise studies at Lahore[16] and Karachi[17]. But, uric acid was significantly present in mixed stones, 25.6% in this study, like 48.5% in Khan’s study[16] and 59.7% in Rizvi’s study[17]. Absence of pure uric acid stone may be due to less number of study subjects in these studies. Whereas, homogenous uric acid stones was the commonest variant (28.1%) in Multan[9], 6.5% in Pakistan[1],19% in Saudi Arabia[7], 16.2% in Iran[1,7] and 9.5% in France[10]. So, marked variability was noticed in the frequency of pure uric acid stones in the studies from different countries and even in different studies from the same country. Such stones are common in individuals with hyper- uricemia, such as patients with gout and diseases involving rapid cell turnover, e.g. leukemia. However, more than half of all patients with uric acid calculi have neither hyperuricemia nor increased urinary uric acid excretion. In this group, low urinary pH below 5.5 predisposes renal stone formation, because uric acid is insoluble in acidic urine hence tends to precipitate[15]. Again high animal protein consumption and affluence may be one of the reasons of uric acid stone formation[7,8,10].

So, calcium oxalate in pure homogenous form or in combination with calcium phosphate or urate or struvite appears to be the most predominant form all around the world. Pure struvite stones are not uncommon ranging from 1.7% to 17.4% found in different studies. Though, in present study 24.3% mixed renal stones had struvite, was not observed in other studies. Again 'Uric acid in mixed stone' was reported in fair number of cases in this study but homogenous uric acid stone was not found in this study. Pure uric acid stones were reported in 0.43% to 28.1% cases in other studies. Cystine is a rare component of renal stone and found only in 'mixed stones' throughout the world.

Conclusion

Renal stone disease is a male predominant health disorder affects mostly young working group. In this study renal stones were analyzed by semi-quantitative chemical analysis, traditionally in use in most of the lab due to its ease though a time consuming technique and necessitate large stone volume. We found mixed stones as the commonest variant and predominant chemical component was Calcium oxalate. FT-IR spectroscopy is considered as the most appropriate technique hence becoming the gold standard in renal stone analysis. So, further studies employing a quantitative technique preferably FT-IR spectroscopy would strengthen the study findings. Besides dietary habit, systemic diseases, urinary tract infection, anatomical abnormality of urinary tract and genetic factors of patients were not considered in this study to correlate chemical composition of renal stones with aetiopathogenesis, so, more researches are needed in these regards in context of Bangladesh.

References

1. Bangash K, Shigri F, Jamal A et al. Spectrum of Renal Stones Composition; Chemical Analysis of Renal Stones. *International Journal of Pathology* 2011; 9(2):63-6.

2. Chand H, Abbasi KA, Shaikh KA et al. Chemical Composition of Urinary Stones, in Stone Passers at Larkana. *Pakistan Journal of Medical and Health Sciences* 2011; 5(2):262-4.

3. Phillip MH. Nephrolithiasis: treatment, causes and prevention. *Cleveland Clinic Journal of Medicine* 2009; 76(10):583–91.

4. Colella J, Kochis E, Galli B et al. Urolithiasis/ Nephrolithiasis: What's It All About? *Urologic Nursing* 2005; 25(6):427-48, 475.

5. Hossain MJ, Uddin MN, Islam MS. Result of ESWL in the management of urinary stones, in the department of urology, Chittagong Medical College Hospital: A study of 500 cases. *JCMCTA* 2009; 20(1):45-9.

6. Risal S, Risal P, Pandeya DR et al. Spectrum of stones composition: a chemical analysis of renal stones of patients visiting NMCTH. *Nepal Medical College Journal* 2006; 8(4):263-5.

7. Shokouhi B, Gasemi K, Norizadeh E. Chemical Composition and Epidemiological Risk Factors of Urolithiasis in Ardabil Iran. *Research journal of Biological Science* 2008; 3(6):620-6.

8. Khan AS, Rai ME, Gandapur PA et al. Epidemiological risk factors and composition of urinary stones in Riyadh Saudi Arabia. *J Ayub Med Coll Abbottabad* 2004; 16(3):56-8.

9. Rafique M, Bhutta RA, Rauf A et al. Chemical composition of upper urinary tract calculi in Multan. *J Pak Med Assoc* 2000; 50:145-8.

10. Gurau G, Dobre M, Paula P et al. Evaluation of the Chemical Composition of Renal Stones. Analele Universitat II "Dunarea de Jos". *Galati Medicina Fascicula* 2014; 17(1):111-8.

11. Farooq M, Hameede A, Anwar M et al. Urinary calculi biochemical profile of stones removed from urinary tract. *Professional Med J* 2007; 14(1):6-10.

12. Prasongwatana V, Bovornpadungkitti S, Chotikawanich E et al. Chemical components of urinary stones according to age and sex of adult patients. *J Med Assoc Thai* 2008; 91(10): 1589-94.

13. Rizvi SA, Sultan S, Zafar MN et al. Evaluation of children with urolithiasis. *Indian J Urol* 2007; 23(4):420-7.

14. Rizvi SA, Naqvi SA, Hussain Z et al. Pediatric urolithiasis: developing nation perspectives. *J Urol* 2002; 168(4):1522-5.

15. Kumar V, Abbas AK, Aster JC. Robbins and Cotran Pathologic Basis of Disease, 9th ed. Saunders Elsevier 2015: 951–2.

16. Khan SI, Saeed M. Observations on the chemical profile of urinary calculi. *The Professionals* 1997; 2:151–4.

17. Rizvi S. A Calculous disease. A survey of 400 patients. *JPMA* 1975; 25:268-74.

18. Altaf J, Arain AH, Kella NL et al. Chemical Analysis of Urinary Stones and its Locations Associated to Urinary Tract. *JPMI* 2001; 15(2):199-201.

19. Ullah I, Alam K, Wazir BG et al. Chemical Composition of Urinary Tract Stones: Experience at a Referral Center of KPK Province. *Ann Pak Inst Med Sci* 2012; 8(1):31-4.