Spectrum of urinary stone composition in Northwestern Rajasthan using Fourier transform infrared spectroscopy

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ABSTRACT

Introduction: The aim of this study was to evaluate the chemical composition of urinary stones and pattern of changes according to the patient’s age in Northwestern Rajasthan using Fourier transform infrared (FTIR) spectroscopy.

Materials and Methods: A prospective study of 1005 urolithiasis patients was carried out in two tertiary care centers from September 2012 to September 2016. Chemical composition of urinary stones was analyzed using FTIR spectroscopy, and a subgroup study based on the patient’s age was done (8–12 years – Group A, 13–18 years – Group B, and >18 years – Group C).

Results: Out of 1005 patients, 59 were in Group A, 104 in Group B, and 842 in Group C. Male predominance was found in all age groups. Mixed composition stones were much more common than pure one (74.83% vs. 25.17%). Overall, combination of calcium oxalate monohydrate with dihydrate was the most common composition (58.0%). Calcium oxalate was the predominant chemical composition in 91.54% of stones, followed by uric acid in 4.28%, struvite in 2.29%, calcium phosphate in 1.49%, and cystine in 0.4%. The proportion of calcium oxalate stone was increasing while that of struvite, uric acid, and cystine stone was decreasing with age. Most of the vesical calculi in pediatric age group (Group A; 8–12 years) patients were made up of combination of struvite, calcium phosphate, and uric acid. A total of 85.11% of staghorn calculi were of oxalates.

Conclusion: In Northwestern Rajasthan, calcium oxalate is the most common composition of urinary stones in all age groups. Mixed stones are more common than pure ones. The incidence of calcium oxalate stone increases while that of struvite, uric acid, and cystine stone decreases with age.

INTRODUCTION

Urolithiasis is a common cause of morbidity and absenteeism from work worldwide. In India, varied stone compositions are found in different regions, but the exact data are lacking because of paucity of large population-based prospective studies.[1] Approximately 80% of adults with urolithiasis have stones that consist predominantly of calcium oxalate and/or calcium phosphate. Struvite stones (magnesium ammonium phosphate) represent 10%–15%, uric acid stones, 5%–10%, whereas cystine stones are rare, 1%–2%.[2,3] In childrens, the composition of urinary stones is somewhat different than that of adults and also continuously changing from the past.[4] In India, Northwest region is the “stone belt” that includes some part of Maharashtra, Gujarat, Punjab, Haryana, Delhi, and Rajasthan.[5,6] The recurrence rate of stones is unacceptably high at 10% after the 1st year of stone presentation, 35% at 5 years, and 50% at 10 years.[7] Urinary stone analysis assists with identification of the possible causes of renal stone formation and when combined with blood, and urine analysis helps in identification of treatable risk factors to decrease recurrence.

Methods used for renal stone analysis include Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction spectroscopy, and inductively coupled plasma mass spectrometry.
crystallography, coherent-scatter analysis, scanning electron microscopy with energy dispersion, thermogravimetry, polarizing microscopy, and wet chemical analysis. Among all these methods, FTIR is a sensitive, reliable, accurate, rapid, and specific method. A study was therefore done to perform chemical analysis of stones to know the stone pattern in different age group patients presenting to our hospital.

MATERIALS AND METHODS

This was a prospective study conducted on 1005 urolithiasis patients from two tertiary care centers in India, between September 2012 and September 2016. The geography, lifestyle, and dietary habits of the population served by these two centers are comparable. Basic demographic data were collected from the patients, and informed as well as written consent was obtained. To analyze the variability in stone composition in pediatrics and adults, patients were divided into three age groups; 8–12 years (Group A), 13–18 years (Group B), and >18 years (Group C).

The stones recovered from surgery were washed with distilled water to remove blood and tissue remains attached, completely dried with filter paper, and then cut for microscopic structural analysis. To prepare the renal stones for analysis, they were grinded to a fine consistency (using a mortar and pestle) and then dried in a hot air oven for 24 h at 100°C. Then to make pellet, 0.003 mg of the dried powder was mixed with 0.097 mg of potassium bromide powder. This mixture was transferred to the mini hand press to form a pellet which was then transferred onto the spectrophotometer pellet holder for analysis.

FTIR spectroscopy was performed using a Perkin-Elmer Fourier Transformer Infrared Spectrophotometer, with a wave number range from 7800 to 350 cm⁻¹. The instrument had a high sensitivity with a signal/noise ratio of 30,000:1. The spectrum obtained was then compared to the 1668 spectra in the NICODOM-IR kidney stone library.

Pure stones in our study were defined as stone composed of ≥90% of the reported composition. Mixed stones were made up of more than one composition, but none of the compositions was ≥90% of the total. The stone component was considered as predominant one if it exceeded 50% of the total composition of the calculus.

RESULTS

Total 1005 patients with urinary stones were analyzed during September 2012–September 2016. Out of these, 714 were male and 291 were female with the ratio of 2.45:1. There were 59 patients in Group A, 104 in Group B, and 842 patients in Group C. The patient's age ranged from 8 to 72 years. The mean age, male to female ratio, and other demographic parameters are described in Table 1. Urinary stones were more common in males in all age groups, and this male dominance was increasing with age [Table 1].

Out of 1005 stones analyzed, 664 were renal, 219 were ureteric, and 122 were urinary bladder stones. Stones with mixed composition were much more common than pure one (74.83% vs. 25.17%) [Table 2]. Overall the most common composition was the combination of calcium oxalate monohydrate and dihydrate (58.0%) [Figure 1a and b]. Calcium oxalate as predominant chemical composition was present in 91.54% of the stones, followed by uric acid in 4.28%, struvite in 2.29%, calcium phosphate in 1.49%, and cystine in 0.4%. Renal, ureteric, and urinary bladder stones were predominantly calcium oxalate-containing stones in 96.1%, 94.1%, and 62.3%, respectively.

On subgroup analysis according to the age, we have found that renal stones were more common in adults whereas vesical calculus was more common in pediatric patients [Table 1]. Furthermore, the proportion of calcium oxalate stone was increasing while that of uric acid, struvite, and cystine was decreasing with age in all parts of the urinary tract [Table 2]. Calcium oxalate was the most frequent composition in renal and ureteric stones in all age groups, but this was not true for vesical calculus. In Group

| Table 1: Demographic characteristics of patients |
|-------------------------------------------------|
| Demographic variable                           | Age          | Total       |
|                                                 | 8-12 years Group A | 13-18 years Group B | >18 years Group C |
| Sex distribution (%)                           |              |             |                |
| Male                                            | 35 (59.3)    | 71 (68.3)   | 608 (72.2)    | 714 (71.0)   |
| Female                                          | 24 (40.7)    | 33 (31.7)   | 234 (27.8)    | 291 (29.0)   |
| Male:female ratio                               | 1.5:1        | 2.15:1      | 2.60:1        | 2.45:1       |
| Age (mean, years)                               |              |             |                |
| Male                                            | 10.3         | 16.4        | 35.8          | 32.6         |
| Female                                          | 10.6         | 15.8        | 39.6          | 34.5         |
| Location of stone (%)                           |              |             |                |
| Renal                                           | 28 (47.5)    | 64 (61.6)   | 572 (67.9)    | 664 (66.1)   |
| Ureteric                                        | 13 (22.0)    | 20 (19.2)   | 186 (22.1)    | 219 (21.8)   |
| Vesical                                         | 18 (30.5)    | 20 (19.2)   | 84 (10.0)     | 122 (12.1)   |
C, calcium oxalate was the most frequent composition whereas only 33.3% vesical calculi in Group A patients had calcium oxalate as predominant chemical composition while rest were composed of varying amount of struvite, calcium phosphate, and uric acid. A total of 85.11% (143 out of 168) of staghorn calculi were of oxalates followed by varied composition of calcium oxalate, phosphate, struvite, and uric acid (12.51%). Staghorn stones of pure struvite composition were present only in 2.38%.

### DISCUSSION

Northwest Rajasthan is one of the stone belt regions of India. Stone analysis will give a rough idea about the etiology that will help in formulating preventive measures. Both European as well as American urological societies also recommend for stone analysis at least once.

Currently, multiple modalities are available for stone analysis. However, we have chosen FTIR spectroscopy because of quick examination, moderate cost, the ability...
Urinary calculus disease usually occurs in working age group and leads to an economic burden on the society. Jindal et al. in their study showed a mean age of 38 years for urolithiasis. In our study, the mean age was 32.6 years for males and 34.5 years for females. Bangash et al. reported the mean age of 43.9 years, with insignificant sex difference. Males were found to have more stone disease in our study than females with the ratio of 2.45:1, and this male dominance was increasing with age (Group A 1.5:1, Group B 2.15:1, and Group A 2.6:1). This was consistent with most of the other studies.

Urinary stone composition varies across the world and calcium oxalate stones are found to be the most predominant composition. Pediatric patients show different stone profile than adults. Very scanty data of pediatric stone composition are available in Indian literature. Ansari et al. analyzed 1050 urinary stones recovered from patients in Northern India and found that calcium oxalate was the most predominant component present in 93.04% of the cases followed by struvite in 1.42%. Similar results have been reported from different parts of India. Gabrielsen et al. while analyzing the data of 5245 stones belonged to 1–18 years old patients of the United States reported calcium oxalate as the most common stone composition. We have also found calcium oxalate as the most common composition and was present predominantly in 91.54% of stones followed by uric acid in 4.28%, struvite in 2.29%, calcium phosphate in 1.49%, and cystine in 0.4%. This pattern of calcium oxalate as predominant composition was seen in all age groups (Group A 57.6%, Group B 81.7%, and Group C 95.1%) [Table 4].

The high incidence of calcium oxalate stones in our region may be because of its hot climate, oxalate-rich diet, and decreased fluid intake. On subgroup analysis based on the patient’s age, we observed that the proportion of calcium oxalate stone was increasing whereas that of uric acid, struvite, and cystine was decreasing with age, and this trend was similar for all whether it was renal, ureteric, or vesical calculus. Gabrielsen et al. reported similar trends while evaluating the stone composition in pediatric patients in a large retrospective study. Other studies also showed peak incidence of calcium oxalate stones between the age of 30 and 50 years.

### Table 3: Stone analysis techniques - pros and cons

| Technique                  | Advantages                                      | Disadvantages                                      |
|----------------------------|-------------------------------------------------|---------------------------------------------------|
| Fourier transform infrared spectroscopy | High sensitivity - can examine small amount of sample | Lengthy and time-consuming preparation |
|                            | Fast result                                     | Because of overlapping absorption bands, sometimes there is difficulty in detection of small amounts of components in some complex stones |
|                            | Cost effective                                  | Needs large sample size                            |
|                            | Does not need highly qualified technician       | Time taking                                       |
|                            | Semiautomatic evaluation and able to identify organic components or noncrystalline substances | Gives information about individual ions and radicals rather than a specific compound |
|                            | Can also recognize even small fractions of multiple components as percentage more precisely and accurately as compared to other techniques | Cannot detect amorphous or noncrystalline components |
|                            | Simple to perform                               | Costly                                            |
| X-ray diffraction          | Analysis is quantitative                        | Needs qualified technicians                        |
|                            | Can be applied on small sample size             |                                                  |
|                            | Measurement is automatic                        |                                                  |
|                            | Easy                                            |                                                  |
|                            | Exact differentiation of all crystalline components is possible |                                                  |
| Scanning electron microscopy | Possibility to visualize the components, their shape, internal structure, location inside the stone, and relation between the crystals and the organic matrix |                                |
|                            | Simple                                          |                                                  |
|                            | Fast                                            |                                                  |
| Thermogravimetry           | Simple                                          |                                                  |
|                            | Fast                                            |                                                  |
|                            | Closely related compounds are difficult to differentiate |                                                  |
|                            | Needs large amount of sample                    |                                                  |

### Table 4: Distribution of stones having calcium oxalate as predominant composition

| Location | 8-12 years Group A (n=59), n/total (%) | 13-18 years Group B (n=104), n/total (%) | >18 years Group C (n=842), n/total (%) | All age groups (n=1005), n/total (%) |
|----------|----------------------------------------|------------------------------------------|----------------------------------------|--------------------------------------|
| Renal    | 20/28 (71.4)                          | 58/64 (90.6)                            | 560/572 (97.9)                        | 638/664 (96.1)                      |
| Ureteric | 8/13 (61.5)                           | 17/20 (85.0)                            | 181/186 (97.3)                       | 206/219 (94.1)                      |
| Vesical  | 6/18 (33.3)                           | 10/20 (50.0)                            | 60/84 (71.4)                         | 76/122 (62.3)                       |
| Any location | 34/59 (57.6)                     | 85/104 (81.7)                           | 801/842 (95.1)                      | 920/1005 (91.5)                     |
Pure uric acid stone was present in 13.65% of patients in Group A in contrast to only in 0.8% of patients in Group C. Urinary uric acid excretion rates decrease with age in pediatric patients and might impact on stone formation.24 Struvite stones are the best marker of urinary tract infection. The incidence of struvite and calcium phosphate stones is decreasing because of early detection and vigorous treatment of urinary tract infection with antibiotics nowadays. Due to more chances of urinary tract infection in uncircumcised males and young females, these stones were more common in Group A than in Group C. Alaya et al. and Daudon et al. also found decreasing frequency of struvite and calcium phosphate stones with age.14,23 The fact that no cystine stones were detected in Group C may be because the formation of cystine stone is genetically determined and most patients present in childhood.

In the past, struvite was the main component of staghorn calculus, but now, calcium oxalate predominates in staghorn stones.1,16,25,26 In the present study, 143 out of 168 (85.11%) staghorn calculi were made up of oxalates followed by mixed composition in 12.51% as the second most common composition. Rest of 2.38% staghorns were made up of struvite. Again, the frequent use of antibiotics for urinary tract infection appears the reason of this changing trend.

The composition of vesical calculus usually differs between pediatric and adult patients. Vesical calculus in children is usually related to poor nutrition or urinary tract infection while in adults and old age patients, it is mostly because of bladder outlet obstruction. In our patients, calcium oxalate was the most common composition in vesical calculus in adults, but in pediatric patients of 8–12 years age, mixed stones containing struvite, calcium phosphate, and uric acid were the most common composition [Table 4].

CONCLUSION

The present study concludes that in Northwestern Rajasthan, calcium oxalate is the most frequent composition of urinary stones in all age groups. The incidence of calcium oxalate stone increases while that of struvite, uric acid, and cystine stone decreases with age. Further studies are needed to evaluate the mechanism behind these differences to decrease the incidence, prevalence, and recurrences of urinary stone disease.

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