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Mineral composition of urinary stones- Quantitative analysis by FTIR Spectroscopy

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ABSTRACT

Urinary stones are mineral material deposition within the urinary tract. Aim of this study is to find out mineral composition of urinary stones which can guide treatment and give information that may prevent recurrence of stones. This study was conducted in the Department Urology and Biochemistry, Yenepoya Medical College Hospital, Mangalore, India during the period December 2011 to October 2013 and the quantitative mineral composition of stones were assessed by Fourier Transform Infrared Spectroscopy (FTIR). Approximately 79.5% of stones were mixed and 20.5% of stones were pure stones. FTIR data revealed that mixed stones were primarily composed of calcium oxalate (90.5%), calcium phosphate (67%) and uric acid 25% and small percentages of calcium carbonate and magnesium ammonium phosphate.75.6% of pure stones were composed of calcium oxalate, 19.5% of uric acid stones and 4.9% calcium phosphate stones

KEYWORDS Urinary stones, Urolithiasis, Fourier Transform Infrared spectroscopy, FTIR, Calcium oxalate stones.

Introduction:

Urinary stone analysis is the test done on the stones formed in the urinary tract which can cause severe pain and even renal damage.The basis of formation of these stones is the change in the normal balance of water, salts, minerals and other substances found in urine. Important factor is water deficiency, which causes aggregation of the salts, minerals, and other substances in the urine that precipitate formation of stones [1].

It is very important to know the chemical composition of stone, which can guide treatment and give information that may prevent recurrence of stones. Urinary stones may contain various combinations of chemicals in different proportions; the most common type contains calcium in combination with either oxalates or phosphates. A less common type of stones are uric acid stones, struvite and cystine stones[2].Hypocitraturia and hyperoxaluria are the common abnormalities found in the urine of stone formers. Stone formers have significantly higher urinary oxalate, calcium, uric acid and hypocitraturia depending on the type of stones formed in them than normal individuals[3].

The constituents of urinary stones may be classified by different analytical techniques, e.g. wet chemical methods or by instrumental techniques such as crystallographic methods [4], thermal analysis[5], FTIR and Raman spectroscopy[6].The widespread acceptance of the infrared spectroscopy as an analytical research tool in many industrial laboratories suggested its use as a new method for the analysis of urinary calculi[7], and this has accepted as a valuable quantitative method for renal stone analysis in the last decade[8]. Infrared spectroscopy has already been used successfully in a similar application in the determination of mineral constituents of rocks[9],[10]. The technique is cost effectives and it makes possible to examine quantification of relative amount of constituent presents in the stones. FTIR spectroscopy has been cited to be the most appropriate technique for urinary stone analysis [11].

This study gives useful information regarding quantitative mineral composition of urinary stones and the results of stone composition analysis helps in dietary modification and treatment.

Methods:

This study was conducted in the Department of Urology and Biochemistry in Yenepoya Medical College Hospital, Mangalore, Karnataka State, India during December 2011 to October 2013 after obtaining University ethical committee approval. 200 patients (174 males and 26 females) with urolithiasis underwent endoscopic / open procedures. The age of the subjects ranged from 2 to 80 (mean 43, SD=15.201).

The stones were washed carefully with distilled deionised water and examined for physical characteristics and later dried. The stones were powdered with pestle and mortar. This procedure produced a fine homogenous powder. 100mg of this powder were mixed with potassium bromide and the pellet was subjected to FTIR analysis. The FTIR spectroscopy was per-

formed using Shimadzu 8700 FTIR Spectrophotometer in the frequency range 600-4000 cm^{-1}.The output graph obtained i.e., percentage of transmission against wavelength were analysed for stone composition comparing with graphs from standard stone library. The Wilcoxon signed-rank test is used to compare two related samples.

Results:
200 urinary stones were analyzed. The prevalence has predominantly high in males, 174 (87%) than 26 females (13%) data shown in Table 1.

The highest number of cases, 43.5% was present in age group of 41-60 years followed by 38% in age group of 21-40. The least number of stone formers were in patients below age of 20(5.5%) years. The highest numbers of males were present in the age group of 41-60 (39.5%) whereas females in 21-40 (7.5%) which as shown in Figure 1.

Amongst the patients calcium oxalate is predominant (90.5 %) however 31 cases (15.5%) were pure calcium oxalate stones of which 28 stones (90.3%) were monohydrate and 3 stones (9.7%) were dihydrate. Out of 200 cases, 12 (6%) stones were mixed calcium oxalate and phosphate stone and calcium oxalate was the predominant component. Uric acid was present in 42 (21%) cases.4% of uric acid was in pure form. 17% of uric acid stones were mixed with other minerals like calcium oxalate (83.3%) and calcium phosphate (68.2%).Typical FTIR spectra of pure calcium oxalate, uric acid and mixed stones (calcium oxalate and uric acid) are shown in figure 2,3 and 4 respectively. The diagnostic bands identified for calcium oxalate were the strong bands around 2368.45, 986.96, 893.5 cm^{-1}, pure uric acid 1954.10 cm^{-1} and for uric acid + calcium oxalate were around 2365.93, 1011.53, and 904.58 cm^{-1}respectively. Calcium carbonate was measured using the peaks at 1403 and 855 cm^{-1}.

There is a statistically significant difference between calcium oxalate monohydrate and calcium oxalate dihydrate ($P<0.001$), and calcium phosphate and calcium carbonate ($P<0.001$)

Discussion: The information of chemical composition of urinary stones is essential for knowing their etiology. The therapy of urinary stone disease to some extend can be based on chemical analysis of calculi. Recurrence of stone formation can be prevented by knowledge of the chemicals responsible for stone formation [12].

According to our study the urinary stones are more common in males than females with a ratio of 6.7:1. This data is comparable to the study by Ghazi Khan et al[13]who found male to female ratio of 7.8:1 and with Rajput et al.,[14]who found male to female ratio of 4:1. Our findings also correlates with the previous finding of Pandeya[15] and Stapleton[16]. This may be because of the larger muscle mass of men as compared to women. The daily breakdown of the tissue results in increased metabolic waste and a predisposition to stone formation. The other significant cause may be because of the male urinary tract being more complicated than the female urinary tract.

In our study the prevalence of urinary stones was highest in the age group of 41-60 years 43.5%, followed by 21-40 years 38 %. It was less common in age group of above 60 years 13%, and the prevalence was found to be least in 01-20 years 5.5%. This is comparable to study conducted by Ahmad et al[17] whose study shown maximum incidence in age group 30 to 50 years while the study by Rajput et al [14] showed that urinary calculi are more prevalent in age group between 21 to 40 years. Some studies have stated that stones are more prevalent when men enter 40s and the risk of stone formation as they enter into the 70s. For women, the prevalence peaks in their 50 years [18].

In our study pure calcium oxalate stones were present in only 21.5% but calcium oxalate was the predominant constituent in all the mixed stones. Also our results shows that 69.5% stones were mixed calcium oxalate stones .In a study by Sial et al,[19] the pure calcium oxalate stones were 5 (10%), calcium oxalate plus calcium phosphate were 10 (20%) and stones having uric acid in mixed form were 60%, although sample size was small (n=50). These results are very similar to our study.

In a study reported by Rizvi et al [20] showed that, the commonest stone were calcium oxalate followed by calcium oxalate plus uric acid and calcium phosphate. No pure uric acid or ammonium urate calculi were reported. Our study showed that 25% of the calculi contained uric acid either mixed orin pure form. Study by Zafar et al[21] and Khalil et al [22] reported that, 43.3% and 40.8% of the calculi contain uric acid stones respectively. These results are almost similar to our study.

Most stones are of mixed composition and about 80% are made of a mixture of calcium oxalate and calcium in various proportions. The presence of unique, but unusual compound defines a specific type of urolithiasis. Quantitative evaluation of components is needed to provide full information. Stone analysis by FTIR serves as definitive method for analysis of composition of stone.

FTIR has been suggested to be efficient and accurate technique to determine urinary stone composition·Using computerized IR spectrophotometer and large reference library enable us to determine exact quantitative stone composition, and this method should be extended to all urolithiasis centers[8]. Reference libraries are commercially available and this will help quick analysis of renal stone composition. The qualitative and quantitative determination of the urinary stones can be easily evaluated by FTIR and also it aids to differentiate monohydrate and dihydrate forms of calcium oxalate, hydroxyl and carboxyl form of calcium phosphate quantitatively. Also the typical amount of sample required is low (10 to 100 mg) as compared to other techniques. According to Rebentisch, X-ray diffraction and IR spectroscopy methods give comparable and highly acceptable analytical results and can be classified as reference methods for the analysis of urinary calculi[23].

Calcium oxalate is the most of predominant organic constituent in most of the urinary stones, because normal urine is frequently supersaturated with respect to calcium oxalate, urinary crystals are often formed[24].Calcium oxalate stones mainly developed due to hyperoxaluria, which is metabolic disorder that causes the stone formation[25]. The present study showed a higher prevalence of calcium containing stones (calcium oxalate monohydrate) as the predominant type (90.5%) which is supported by the study of Pandeya and Asplin[15],[26]. FTIR provided method to differentiate calcium oxalate monohydrate from calcium oxalate dihydrate, which is not possible with conventional chemical analysis. The prevalence of recurrent calcium oxalate stones has progressively increased in untreated subjects, approaching a 50% recurrence rate over 10 years [27].

Conclusions:
Calcium oxalate, calcium phosphate and uric acid containing urinary stones are commonly found in patients with urolithiasis. FTIR Spectroscopy is an efficient and accurate technique to analyze mineral composition of urinary stones and helps to better understand the chemicals responsible for formation and recurrence of renal stones.

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Table 1: Prevalence of stones according to gender

Sex	Frequency	Percentage
Males	174	87
Females	26	13
Total	200	100

Figure 1: Prevalence of stone among different age group

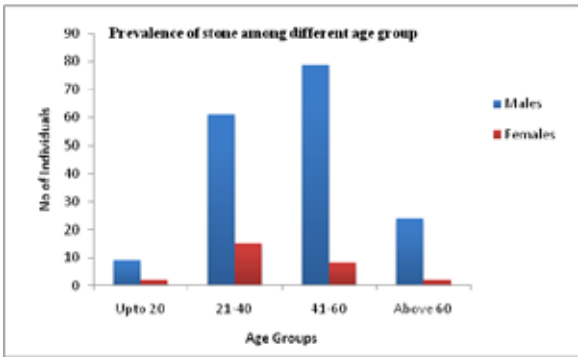


Figure 2: Typical FTIR spectrum of pure calcium oxalate urinary stone sample

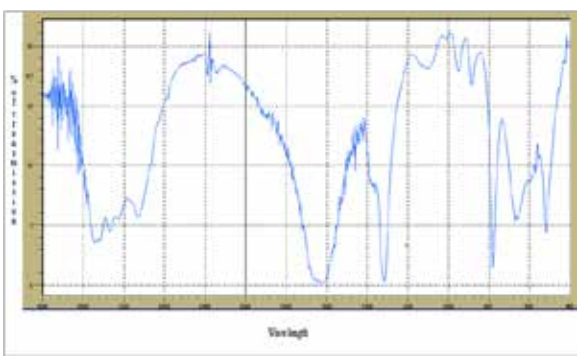


Figure3: Typical FTIR spectrum of pure uric acid urinary stone sample

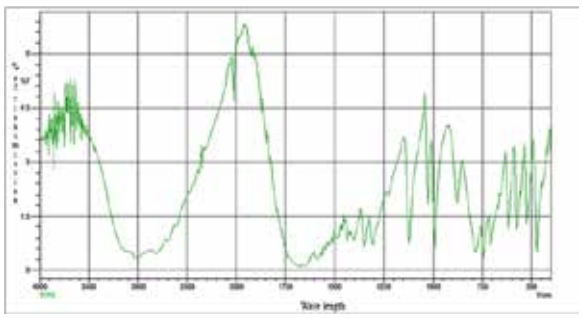
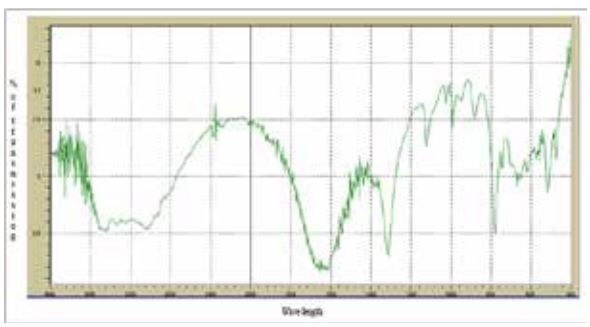


Figure 4: Typical FTIR spectrum of mixed stones (calcium oxalate + uric acid)



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