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To Study the Biochemical Analysis of Kidney Stone by Using FTIR Spectroscopy in the Patients with Renal Calculosis.

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ABSTRACT

Urolithiasis can be defined as the clinical condition where the formation of crystal aggregates in the urinary tract results in kidney stones. The presence of a kidney stone in the human body can be externalized in very different ways, it can produce no symptoms but it can be associated with several pain caused for one or some of the following symptoms: gross or microscopic hematuria, obstruction of one or both kidneys, and urinary infections. In the present study, renal stone analysed in people of Delhi by FTIR which assist the identification of the possible causes of renal stone formation and therefore with the prevention of recurrent stone formation. The result spectroscopy to determine the constituents of the stones calcium oxalate, uric acid and ammonium magnesium phosphate hexahydrate. The present study provides assistance for appropriate treatment of the patient and prevent a recurrence of stone formation in people.

INTRODUCTION

Kidney stone is a solid lump (from as small as a grain of sand to as large as the size of a golf ball) made up of crystals that separate from urine and build up on the inner surfaces of the kidney. Kidney stones result from the precipitation of certain substances within the urine. In some cases, the stone may not be able to travel through the ureter, causing pain and possibly an obstruction, blocking the flow of urine out of the kidney [1,2]. Severe pain or aching in the back on one or both sides, sudden spasms of excruciating pain (renal or uterine colic), bloody, cloudy or smelly urine, feeling of being sick, a frequent urge to urinate, or a burning sensation during urination, fever and chills, etc. are commonly observed symptoms in the patients. Super saturation creates stone by causing ions in solution to combine with one another into a solid phase by nucleation [3,18,19]. Calcium and oxalate ions can orient themselves on surfaces of another crystal, such as uric acid, and such heterogeneous nuclei may promote calcium oxalate stones. Hereditary and personal history of renal stone and geographic conditions also influences geographic conditions also influences stone formation [4,16,17].

Urine analysis, X-ray images, intravenous program and ultrasound were only used till now for diagnosis but now recently introduced non-contrast helical computerized tomography is the first-line investigational tool [5,20]. Presently crystallographic examination is one of the most precise and less expensive methods to identify the nature of the concretion [6]. Thiazide diuretics, allopurinol, etc are used for treatment but they have their own pharmacological limits and number of side effects on long term use [7]. Thus, surgery is the only prime treatment of urolithiasis [8]. After spontaneous passage or surgical treatment, a subset of these patients will have recurrent calculi [9]. These recurrent stone events are significantly morbid and can potentially lead to serious chronic renal disease, thus prevention is a very important treatment goal [10].

Fourier transform infrared spectroscopy is another suitable analytical technique from which information about the composition of kidney stones could be achieved rapidly [11,12,13]. Furthermore, the quantification of the relative amount of each constituent present is possible without using any solvent [14,15]. Therefore, FT-IR spectroscopy has been supposed to be the most appropriate technique for kidney stone analysis and therefore with the prevention of recurrent stone formation [21].

MATERIALS AND METHODS

Sample Material

Careful sample preparation is a key issue in kidney stone analysis. The kidney stones for the present study were surgically removed from 500 patients. All kidney stones after surgery were placed on sterile gauze to dry in air and washed with distilled deionised water to remove the bile and debris. After drying, the kidney stones were cut into small pieces to get representative samples. One quarter portion of the total kidney stone size was grounded with a pestle and mortar until a fine homogeneous powder was obtained, which was then stored in a glass tube, and kept in a dark cabinet until analysis.

Standards

Pure standards of calcium oxalate mono and dihydrate, calcium phosphate ammonium hydrogen urate, uric acid anhydrous, cholesterol and ammonium sodium urate were obtained from Merck company. Initially, the FT-IR spectra of all individual standards were acquired. NICOLET 6700 IR Kidney Stone Library containing 1668 transmission FT-IR spectra was installed within the software of FT-IR. Then spectra of each individual and combined standard (mixed with different ratios of most commonly found kidney stones such as oxalates, uric acid and phosphate) were matched with the spectra stored in the library. However, our own spectral library was also developed to differentiate the kidney stone samples with their matching percentages.

Preparation of the pellets

The concentration of the potassium bromide in sample should be in the range of 0.2% to 1%. The pellet is much thicker than a liquid film, hence a lower concentration in the sample is required. Too high a concentration usually causes difficulties obtaining clear pellets. The IR beam is absorbed completely, or scattered from the sample which results in very noisy spectra. The finely powdered potassium bromide will absorb more humidity (it is hygroscopic) from the air and therefore lead to an increased background in certain ranges. Therefore dried FTIR grade potassium bromide was transferred from the oven into a mortar. 1 to 2 % of powdered calculi sample was mixed and again grounded in to a fine powder. Two stainless steel disks were taken out of the desiccators. A piece of the precept cardboard was placed on the top of one disk and the cut out hole was filled with the finely ground mixture. The second stainless steel disk was put on the top and the sandwich was transferred onto the pistil in the hydraulic press. With a pumping movement, the hydraulic pump handle was moved downward. The pistil started to move upward until it reached the top of the pump chamber. Then, the pump handle was moved upwards and pumped until the pressure reached 20,000 prf. It was left as such for a few seconds and with the small lever on the left side, finally the pressure was released and the disks were removed and pulled apart. The film was removed which was homogenous and transparent in appearance. The pellet of homogenous calculi and potassium bromide were obtained. The pellets were placed in the transmission holder in front of the IR beam. The spectra were acquired in the middle IR range, i.e. 400 to 4000 cm^{-1}

Instruments

FTIR spectroscopy has being used extensively for the identification of organic and inorganic compounds. FTIR spectroscopy has been often used to study urolithiasis. This technique is specific, rapid and versatile and thus can be used for analysis of urinary stone. FTIR spectroscopy provides qualitative and semi-quantitative analysis. Depending on the chemical bonding conditions and also on the particular structure, every molecule gives a characteristic absorption spectrum in the infrared region. The vibration motions of atoms in bonds (bond stretching/ contracting/ bond wagging) are measured by passing FTIR through the powdered stone sample, which has been compressed into a nearly transparent wafer with adequate quantity of potassium bromide. In the FTIR region (4000-200 cm^{-1}), oscillations can be induced in molecules and crystals which bring about an alteration of the dipole moment of the oscillating system.

Chemicals

Pure calcium oxalate, potassium bromide, pure uric acid chemicals and reagents used were of analytical grade.

Sample

Surgically remove kidney stone samples

FTIR spectral measurements

Analysis of the constituents in urinary calculi using FTIR spectrum was made for all the five hundred samples. Out of which 392 samples were found to be a mixture of calcium oxalate monohydrate and calcium phosphate. The important spectral characteristics of pure COM (calcium oxalate monohydrate) are that it shows grouping of five bands between 3477–3047 cm^{-1} , which is due to symmetric and asymmetric O-H stretch. Absorptions at 1620 cm^{-1} and 1320 cm^{-1} may be due to vibration of C=O and C-O respectively. The absorption band at 885 cm^{-1} is due to C – C stretching mode. The bands at 662 and 781 cm^{-1} are due to the out of plane O-H bending and C-H bending mode respectively and band at 517 cm^{-1} arises due to O-C-O in-plane bending. The discrete formation of the two bands at 780 and 517 cm^{-1} is important for distinguishing COM from COD. Phosphate group usually has an absorption range of 1000-1100 cm^{-1} .

The stones of 24 samples were found to be the combination of calcium oxalate dehydrates (COD), calcium phosphate and magnesium ammonium phosphate hexahydrate. The broad band at 3000 cm^{-1} is due to the presence of O-H group. The absorbance at 1670 and 1348 cm^{-1} shows the presence of C=O and C-O stretching vibrations. The broad band at 3000 cm^{-1} may be due to the dihydrate form of calcium oxalate. The method of distinguishing hydroxyl apatite and carbonate apatite from calcium phosphate spectra of FTIR is mentioned in earlier studies. It was stated that in carbonate containing phosphate ions may substitute Phosphate and OH ions. The carbonate bands at 850, 1414 and 1457 cm^{-1} are characteristic for this kind of phosphate and give only a slight possibility of distinguishing from apatite. It might be possible for pure calcium phosphate stones, but in this study, as calcium phosphate was found as a mixture with magnesium ammonium phosphate hexahydrate and calcium oxalate, the differentiation between hydroxyphosphate and carbonate phosphate was not possible and henceforth it was mentioned only in general as phosphate. Magnesium ammonium phosphate hexahydrate usually associated with stones caused by infection is found to be mixed with phosphate in varying proportions.

Table 1: Characteristic group present with it's FTIR bands observed for different types of renal calculi.

TYPES OF STONE	PRINCIPALE IR BAND OBSERVED	CHARACTERSTIC GROUP PRESENT
Calcium oxalate monohydrate	Grouping of five bands between 3477-3047 cm^{-1} 1620 cm^{-1} 1320 cm^{-1} 885 cm^{-1} 662 cm^{-1} 781 cm^{-1} 517 cm^{-1}	Symmetric and asymmetric OH stretch C=O stretch C-O stretch C-C stretch Out of plane O-H bending Out of plane C-H bending In-plane O-C-O bending
Calcium oxalate dehydrate	Broad band at 3000 cm^{-1} 1620 cm^{-1} 1320 cm^{-1} 885 cm^{-1} 662 cm^{-1}	Dihydrate form of CaOX C=O stretch C-O stretch C-C stretch Out of plane O-H bending
Uric acid	1637.29 cm^{-1} 1018.13 cm^{-1}	C=C stretching N-H stretching
Carbonate appetite	850, 1414, 1457 cm^{-1}	CO ₃ ⁻²
Calcium hydrogen phosphate	1010 cm^{-1}	PO ₄ ⁻³

Struvite has a characteristic infrared spectrum and is easily recognized even in mixed stones both by the position of the strong band at 1010 cm^{-1} , which is due to the absorption of PO₄ group and by the presence of other bands at 2370, 1476, 1435, 872, 761 and 572 cm^{-1} . The bands at 1476 cm^{-1} and 1435 cm^{-1} are due to vibration of NH₄ group. When struvite is in association with apatite, the presence of latter can be deduced by absorption at 600 cm^{-1} and by a shift of the band at 1010 cm^{-1} towards higher frequencies.

RESULT AND DISCUSSION

During the period 1st September to 31 January 2013, 500 renal stones samples were received for analysis by FTIR in the SRL Diagnostic Ltd, of the 392 (78.4%) were received from male patients 81 (16.2%) from female patients and 27 (5.4%) from child patients in table 5.1. The renal stones 38 (8.6%) were received from patients in age group 0-20 years, 117 (23.4%) in the age group 21-30 years, 119 (23.8%) of the stones were received from patients in the age groups 31-40 years, with 70 (17%) patients were 41-50 years, 31 (6%) of the stone age group 51-60 years and 18 (3.6%) of age group 60 above in table 5.2. Table 5.3 illustrates the minerals found in the 42

renal stones used for in this study with FTIR analysis. Pure Calcium oxalate monohydrate was the most common major constituent found in 390 samples (78%), with carbonate apatite a constituent in 53 samples (10.6%). Calcium oxalate dehydrate was a constituent in 43 samples (8.6%) as was uric acid. Ammonium acid urate (ammonium urate) was found to be a constituent in only 8 samples (1.4%). Calcium hydrogen phosphate dehydrate (brushite) as well as cystine were present in 5 sample each (0.2%). Calcium oxalate monohydrate and uric acid appeared as a combination in 43 samples (8.6%) with the combination of calcium oxalate monohydrate, ammonium urate and carbonate apatite found in 20 samples (4.2%). Calcium oxalate monohydrate and calcium oxalate dehydrate in combination with carbonate apatite only in 53 samples (10.6%). Cystine and carbonate apatite in combination were also only found in 1 sample (0.2%).

Table 2: Distribution of renal stones according to different sex (n=500).

GENDER	NO.OF STONE	PERCENTAGE
MALE	392	78.4%
FEMALE	81	16.2%
CHILD	27	5.4%

Table 3: Distribution of renal stones according to age group (n=500).

AGE GROUPS	NO.OF PATIENTS	%PATIENTS OBSERVED
Less than 20	38	7.6%
21-30	117	23.4%
31-40	119	23.8%
41-50	70	14%
51-60	31	6%
61-above	18	3.6%

Table 4: Prevalence of specific mineral combinations in patient samples (n=500).

TYPES OF STONE	NO. OF STONE	PERCENTAGE
Pure calcium oxalate	390	78%
Pure Uric acid	21	4.2%
Calcium oxalate + uric acid	43	8.6%
Calcium oxalate + magnesium ammonium phosphate hexahydrate	24	4.8%
Calcium oxalate +carbonate phosphate	53	10.6%
Uric acid +2,8 dihydroadenine	20	4%
Pure magnesium ammonium phosphate hexahydrate	7	1.4%
Cystine	1	0.2%

DISCUSSION

The incidence of renal stones has been increasing in the past few decades. Up to 5% of the general population is affected by renal stones, with the lifetime risk of renal stones estimated at 8-10%. The worldwide incidence of renal stones has also been increasing in the adolescent population, with a 4% per year increase in incidence in children of age group 12-17 years. Renal stones are found to be more common in males than in females, with the risk of developing a renal stone in the United States at 12% in men and 6% in women. This was confirmed by a study in South Australia where they found that 70% of all renal stones were in men. The incidence of renal stones in South Africa appears to be similar to that in other western countries.

The age at which renal stones develop varies, but the majority of patients presenting with renal stones are between 20-49 years old. In a study in the US the incidence in men increases after age 20 and seem to peak between 40-60 years. Women, on the other hand, have an increased incidence in their late 20s after which a decrease in incidence occur up to the age of 50 years. In our study we found the majority of stones (44%) to be in patients aged 31-60 years, which is similar to the age groups found in other studies. The majority of stones (20%) were found in men between the ages 41-60 years. The incidence in men peaked at age group 51-60 years and then declined. Women, on the other hand had a peak in incidence at age group 41-50 years which is slightly younger than in men. Only 16% of the renal stones in women were found above the age of 60 years whereas 3% of stones in men were at age above 60 years.

According to literature, the majority of renal stones (78%) contained calcium in combination with either oxalate or phosphate, whilst 4.2% of renal stones contain uric acid. Infection-induced renal stones consist of ammonium magnesium phosphate and carbonate phosphate are found in approximately 10.6% of patients with renal stones. Several risk factors have been identified for the development of renal stones, including water hardness, sunlight and heat, dietary consumption of animal protein, increasing body size. Increased incidence in men may be due to increased intake of dietary animal protein, while endogenous estrogen and estrogen therapy in postmenopausal women was reported to decrease the risk of stone recurrence by decreasing the saturation of urinary calcium and calcium oxalate. The most common metabolic disorder to cause calcareous stones is hypercalciuria. In our study, 392 samples contained calcium in combination with either oxalate or phosphate as one of the constituents.

All of the renal stones analyzed contained more than 1 constituent. Calcium oxalate monohydrate in combination with uric acid was found in 43 (8.6%) samples, whilst in 53 (10.6%) samples it was found in combination with ammonium urate and carbonate apatite. The most common identifiable cause of calcium renal stones is hypercalciuria. The latter occurs in 5-10% of the population, and therefore, calcium in some form is usually present in most renal stones. Different combinations of the other minerals then accumulate in the 'crystal' (renal stone), depending on the cause for the renal stone formation.

Analytical method for the FTIR method is an important process to verify that this analytical method used for renal stone analysis is suitable for its intended use, and to ensure that accurate results are reported. Accurate results are important for the proper management of patients presenting with renal stones and therefore for the prevention of recurring renal stones in these patients. During the process patient samples and pure chemicals were repeatedly analyzed, the results of all the runs compared and this was an indication of the precision of the method. Accuracy and precision are both essential in any analytical method to ensure correct results.

CONCLUSION

Calcium oxalate and carbonate phosphate stones are commonly found in patients of Delhi and adjoining areas. Analysis of risk factors in people of Delhi suggested that 20 to 40 years age group was having high risk of kidney stone formation. Men (78%) were more susceptible to kidney stone formation than women (16.2%). People drinking more water per day were less in the kidney stone patients. Less water consumption was one of the risk factor. Food which increases kidney stone formation like peanuts, whole potato, tea and peanut oil is exceedingly consumed in Delhi. These may lead to kidney stone formation. Determination of composition of stones by FTIR spectroscopy techniques enlighten that stones recovered from the patients of Delhi region were calcium oxalate in majority (78%). It was found that relatively low percentage (4.2%) of patients developed uric acid stones. In conclusion, renal stones are more likely to occur between ages 30 and 60 years with the most likely component to be calcium, in combination with either oxalate or phosphate. The incidence in men is much higher than in women.

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