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UROLITHIASIS AND FTIR SPECTROMETRY

Dr. Syed Mohamed Ibrahim Sulthan
(Ph.D., from Delhi University & Working at Shaqra University)
Bio-Medical Engineering Laboratory,
College of Engineering, Shaqra University,
Ad-Dawadimi, Riyad Province, Saudi Arabia
syed.mohamed@su.edu.sa

Dr. Shabab Ajami Ai-Hammadhi
Dean, College of Engineering, Shaqra University,
Ad-Dawadimi, Riyad Province, Saudi Arabia

ABSTRACT

The clinical specification where the development of crystal aggregate within the tract ends up in urinary organ stones is known as Urolithiasis. Kidney stone generate no symptoms however it may be related to different kind of pain caused for one or a number of the following symptoms: gross or microscopic hematuria, obstruction of 1 or both kidneys, and urinary infections, and the presence of a urinary calculus within the human body is identified in so many alternative ways. In the current study, urinary organ stone analysed in individuals of Saudi Arabia by FTIR that assist the identification of the attainable causes of urinary organ stone formation and so with the hindrance of perennial stone formation. The result spectroscopic analysis to see the constituents of the stones calcium oxalate, uric acid and ammonium magnesium phosphate hexahydrate. This study provides help for applicable treatment of the patient and stop a return of stone formation in individuals.

Keywords— Urolithiasis, urinary organ stone, flow of excrement, flaming commotion, calcium oxalate.

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I. INTRODUCTION

Kidney stone could be a solid lump (from a grain of sand to the dimensions of a golf ball) prepared of crystals that detach from urine and build on the inner surfaces of the excretory organ. Excretory organ stones result from the precipitation of bound substances in the urination. In some cases, the stone might not be ready to travel through the duct, inflicting pain and probably an obstruction, block the flow of excrement out of the kidney [1] [2]. Normally determined symptoms in the patients with kidney stones are, severe pain or aching in the back on one or either side, abrupt spasms of torturing pain (renal or uteric colic), bloody, cloudy or foul-smelling urine, feeling of being unwell, a repeated urge to urinate, or a flaming commotion throughout evacuation, fever and chills, etc. Super saturation produces stone by inflicting ions in solution to mix with each other into a solid section by nucleation [3] [18] [19]. Heterogeneous nuclei might promote calcium oxalate stones, when calcium and oxalate ions will orient themselves on surfaces of another crystal, like uric acid. Hereditary and individual history of urinary organ stone and geographic conditions additionally influences stone formation [4] [16] [17]. We recently launched non-contrast helical computerized tomography is the first-line investigational tool though we performed excretion analysis, X-ray images, intravenous program and ultrasound were so far used for diagnosis [5] [20]. Presently crystallographic examination is one in all the foremost precise and fewer valuable methods to identify the character of the concretion6. Water pill diuretics, Zyloprim, etc are used for treatment but they have their own medical specialty limits and vary of aspect effects on future use [7]. Thus, surgery is that the entirely prime treatment of urolithiasis [8]. Once spontaneous passage or surgery, a group of these patients will have continual calculi [9]. These continual stone events square measure significantly morbid and will likely cause serious chronic internal organ malady, thus bar may be a vital treatment goal [10].

Fourier rework spectrum analysis is another applicable analytical technique from that data regarding the composition of internal organ stones may be achieved rapidly [11] [12] [13]. Moreover, the quantification of the amount of each constituent gift is possible whereas not exploitation any solvent [14] [15]. Therefore,

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principal satisfactory procedure for kidney stone analysis and so with the bar of repeated stone development is FT-IR spectroscopic analysis [21].

II. MATERIALS AND METHODS

i. Sample Material

Careful sample preparation could be a key issue in calculus analysis. The excretory organ stones for the current study were surgically aloof from fifty patients. All excretory organ stones once surgery was placed on sterile gauze to dry in air. To get rid of the digestive juice and debris, it was washed with distilled de-ionised water. Once drying, the excretory organ stones were split into tiny items to urge representative samples. One quarter portion of the whole calculus size was grounded with a pestle and mortar till a fine uniform powder was obtained, that was then keep in a very glass tube, and unbroken in a very dark cupboard till analysis.

ii. Standards

Pure standards of calcium phosphate ammonium hydrogen urate, cholesterol and ammonium sodium urate, calcium mono oxalate and dihydrate, uric acid anhydrous, were obtained from Merck Serono, Jeddah, KSA. Individual standards of FT-IR spectra were acquired initially. During installation of software, NICOLET 6700 IR Kidney Stone Library containing 1668 transmission FT-IR spectra was installed. The spectra stored in the library were matched with the spectra of each individual and combined standard (mixed with various ratios of frequently found kidney stones such as phosphate, uric acid and oxalates). To differentiate the calculus samples with their matching percentages own spectral library was conjointly developed.

iii. Pellets Preparation

The potassium bromide in sample ought to be within the limit of 0.2% to 1% in concentration. The pellet is way thicker than a liquid film, therefore a lower concentration within the sample is needed. Too high a level sometimes causes difficulties getting clear pellets. The IR beam is absorbed fully, or scattered from the sample which ends up in terribly droning spectra. The finely small-grained salt can absorb a lot of

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wetness (it is hygroscopic) from the air and thus result in an exaggerated background in bound ranges. Thus dried FTIR grade salt was transferred from the oven into a mortar. One to two chronicles of finely grained calculi sample was mixed and once more grounded in to a fine powder. Out of the desiccators two stainless steel disks were taken. The cut out hole was stuffed with the finely ground mixture and portion of the precept cardboard was placed above one disk. By placing the second stainless steel disk on the apex and the sandwich was transferred onto the pistil in the hydraulic press. With a pumping movement, the pump handle was emotional downward. The reproductive structure began to move upward till it reached the highest level of the pump chamber. Then, the pump handle was enraptured upwards and pumped up till the pressure reached twenty thousand prf. it had been left intrinsically for many seconds and with the tiny lever on the left aspect, finally the pressure was discharged and also the disks were removed and force apart. The clear homogenous and transparent film was removed. The pellet of consistent calculi and potassium bromide were obtained. The pellets were placed within the transmission holder ahead of the IR beam. The spectra were recorded within the middle IR region, i.e. 400 to 4000 cm^{-1}

iv. Instruments

FTIR spectrographic analysis has being employed extensively for the identification of organic and inorganic compounds. FTIR spectrographic analysis has been usually accustomed study urolithiasis. This method is precise, fast and versatile and so is often used for analysis of urinary stone. FTIR spectrographic analysis provides qualitative and semi-quantitative analysis. Based on the chemical bonding conditions and additionally on the specific structure, each molecule provides a characteristic spectrum within the infrared region. By passing FTIR through the fine-grained stone sample that has been compressed into an almost transparent wafer with adequate amount of potassium bromide will provide the vibrational motions of atoms (bond wagging/ bond stretching/ contracting). Oscillations are often evoked in molecules and crystals that bring forth an alteration of the dipole moment of the oscillatory system within the FTIR region (4000-200 cm^{-1}).

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v. FTIR spectral measurements

FTIR spectrum was created for all the five hundred samples, by analyzing the constituents in urinary calculi. It is found that a combination of calcium oxalate monohydrate and calcium phosphate in 392 samples out of 500. The crucial IR spectral distinctiveness of untainted calcium oxalate monohydrate are that it shows grouping of 5 bands between 3477–3047 cm^{-1}, that is because of symmetrical and asymmetrical O-H stretch. Absorptions at 1620 cm^{-1} and 1320 cm^{-1} is also as a result of vibration of C=O and C-O correspondingly. The optical phenomenon at 885 cm^{-1} is owing to C – C stretching mode. The bands at 663 and 782 cm^{-1} are as a result of the out of plane O-H bending and C-H bending mode correspondingly and band at 517 cm^{-1} arises attributable to O-C-O in-plane bending. The distinct formation of the 2 bands at 780 and 517 cm^{-1} is very important for identifying calcium oxalate monohydrate from COD. An absorption spectrum within the range of 1000-1100 cm^{-1} is associated with Phosphate group.

The stones of twenty four samples be establish to be the mixture of calcium oxalate dehydrates (COD), calcium phosphate and magnesium ammonia phosphate hexahydrate. The broad band at 3000 cm^{-1} is owing to the presence of O-H cluster. The presence of C=O and C-O stretching vibrations are assigned spectral peaks at 1670 and 1348 cm^{-1} shows. The broad band at 3000 cm^{-1} could also be attributable to the dihydrate kind of calcium oxalate. The strategy of identifying hydroxyl mineral and carbonate mineral from calcium phosphate spectra of FTIR is mentioned in earlier studies. it absolutely was expressed that in carbonate containing phosphate ions might substitute Phosphate and hydroxyl ions. The carbonate bands at 850, 1414 and 1457 cm^{-1} are characteristic for this sort of phosphate and provide solely a small chance of identifying from apatite. it would be probable for pure calcium phosphate stones, however during this study, as calcium phosphate was found as a fusion of calcium oxalate and magnesium ammonium ion phosphate hexahydrate, the demarcation between hydroxyphosphate and carbonate phosphate wasn't probable and henceforward it absolutely was mentioned solely in general as phosphate. Magnesium ammonia phosphate hexahydrate typically related to stones caused by infection is found to be mixed with phosphate in variable proportions.

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Struvite contains a characteristic spectrum and is well recognized even in mixed stones each by the position of the sturdy band at 1010 cm^{-1}, that is attributable to the absorption of PO4 cluster and by the occurrence of unusual bands at 572, 761, 872, 1435, 1476, and 2370, cm^{-1}. The bands at 1476 cm^{-1} and 1435 cm^{-1} are attributable to vibration of NH4 cluster. Once srtuvite is in combination with apatite, the existence of latter will be concluded by a shift of the band at 1010 cm^{-1} towards elevated frequencies and spectral absorption at 600 cm^{-1}.

TABLE 1
CHARACTERISTIC GROUP PRESENT IN FTIR BANDS OBSERVED FOR DIFFERENT TYPES OF RENAL CALCULI

Types of stone	**Principle IR Band observed**	**Characteristic group present**
Calcium oxalate monohydrate	Grouping of five bands between	Symmetric and asymmetric OH stretch
	3477-3047 cm^{-1}	C=O stretch
	1620 cm^{-1}	C-O stretch
	1320 cm^{-1}	C-C stretch
	885 cm^{-1}	Out of plane O-H bending
	662 cm^{-1}	Out of plane C-H bending In-plane
	781 cm^{-1}	O-C-O bending
	517 cm^{-1}	
Calcium oxalate dehydrate	Broad band at 1320 cm^{-1}	Dihydrate form of CaOX
	3000 cm^{-1}	C=O stretch
	1620 cm^{-1}	C-O stretch

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	1320 cm^{-1}	C-C stretch
	885 cm^{-1}	Out of plane O-H bending
	662 cm^{-1}	
Uric acid	1637.29 cm^{-1}	C=C stretching
	1018.13 cm^{-1}	N-H stretching
Carbonate appetite	1457, 1414, 850 cm^{-1}	$CO3^{-2}$
Calcium hydrogen phosphate	1010 cm^{-1}	$PO4^{-3}$

III. RESULT

During the period 1st September 2014 to 31 September 2015, 120 renal stones samples were received for analysis by FTIR from Ad-Dawadimi General Hospital, of the 94 (78.3%) were received from male patients 19 (15.8%) from female patients and 07 (5.8%) form child patients in table 2. The renal stones 12 (8.3%) were received from patients in age group 0-20 years, 36 (30.0%) in the age group 21-30 years, 37 (30.8%) of the stones were received from patients in the age groups 31-40 years, with 21 (17.5%) patients were 41-50 years, 10 (8.3%) of the stone age group 51-60 years and 4 (3.3%) of age group 60 above in table 3. Table 4 illustrates the minerals found in the 10 renal stones used for in this study with FTIR analysis. Pure Calcium oxalate monohydrate was the most common major constituent found in 83 samples (71.66%), with carbonate apatite a constituent in 11 samples (9.16%). Calcium oxalate dehydrate was a constituent in 9 samples (7.5%) as was uric acid. Only 1 sample (0.83%) shows that in combination carbonate apatite and Cystine were present 4 samples (3.3%) authenticate incidence of carbonate apatite, ammonium urate and calcium oxalate monohydrate in combination. Exactly two samples (1.66%) shows the presence of Ammonium acid urate (ammonium urate). Cystine and calcium hydrogen were found to be present in two different samples each (0.866%) confirms the existence of phosphate dehydrate. 9

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samples (7.5%) confer the occurrence Calcium oxalate monohydrate and uric acid in combination. 11 samples (9.16%) provides evidence for the presence of carbonate apatite, in combination with calcium oxalate dehydrate and calcium oxalate monohydrate.

TABLE 2

DISTRIBUTION OF RENAL STONES ACCORDING TO DIFFERENT SEX (n=120).

GENDER	NO.OF STONE	PERCENTAGE
MALE	94	78.3%
FEMALE	19	15.8%
CHILD	07	5.8%

TABLE 3

DISTRIBUTION OF RENAL STONES ACCORDING TO AGE GROUP (n=120).

AGE GROUPS	NO.OF PATIENTS	%PATIENTS OBSERVED
Less than 20	12	10.0%
21-30	36	30.0%
31-40	37	30.8%
41-50	21	17.5%
51-60	10	8.3%
61-above	04	3.3%

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TABLE 4

PREVALENCE OF SPECIFIC MINERAL COMBINATIONS IN PATIENT SAMPLES (n=120).

Types of stone	No. of stone	percentage
Pure calcium oxalate	83	71.66%
Pure Uric acid	05	4.16%
Calcium oxalate + uric acid	09	7.5%
Calcium oxalate + magnesium ammonium phosphate hexahydrate	05	4.16%
Calcium oxalate +carbonate phosphate	11	9.16%
Uric acid +2,8 dihydroadenine	04	3.33%
Pure magnesium ammonium phosphate hexahydrate	02	1.66%
Cystine	01	0.83%

IV. DISCUSSION

In the past few decades, the occurrence of urinary organ stones has been increasing. The estimated lifespan risk of renal stones is about 8-10%, and up to 5% of the general population is being affected. Among children in the age group of 12-17, the prevalence of urinary organ stones increasing at the rate of 4% per year and also among the adolescent population worldwide. For example the rate of prevalence of urinary organ stones is 6% in women and 12% in men in United States hence urinary organ stones are more common in males than in females. 70% of all renal stones were in men in South Australia and it is being confirmed by a study and similar results are being reported from South Africa.

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

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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 (71.66%) contained calcium in combination with either oxalate or phosphate, whilst 4.16% of renal stones contain uric acid. Infection-induced renal stones consist of ammonium magnesium phosphate and carbonate phosphate are found in approximately 9.16% 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, 83 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 9 (7.5%) samples, whilst in 11 (9.16%) 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. Correct results are necessary for the appropriate management of patients presenting with urinary organ stones and thus for the deterrence of recurring urinary organ stones in these patients. During the process

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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.

v. CONCLUSION

Calcium oxalate and carbonate phosphate stones are commonly found in patients of Saudi Arabia. Analysis of risk factors in people of Saudi Arabia suggested that 20 to 40 years age group was having high risk of kidney stone formation. Men (78.3%) were more susceptible to kidney stone formation than women (15.8%). 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. These may lead to kidney stone formation. Determination of composition of stones by FTIR spectroscopy techniques enlighten that stones recovered from the patients of Saudi Arabia were calcium oxalate in majority (71.66%). It was found that relatively low percentage (4.16%) 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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