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BLADDER STONE COMPOSITIONAL ANALYSIS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND RAMAN SPECTROSCOPY

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ABSTRACT

Introduction - Urolithiais is indeed a significant health problem. Recurrence of Urolithiasis can be prevented by knowing the composition of the stone. **Aim** - The aim of the study is to determine the mineralogical content of bladder calculi by Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy in the Indian subpopulation. **Materials And Methods -** 75 Urinary Bladder Stones were collected post Lithotripsy and were washed with distilled water, dried and further analysed. These stones underwent Raman and IR spectral analysis. Two samples has been explored using Powder X-Ray diffraction (PXRD), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), Total reflection X-Ray Fluorescence Spectroscopy (TXRF) and Infrared (IR) chemical mapping techniques to demonstrate their ability to be in agreement with IR and Raman spectral analysis of 75 samples. **Results –** Among 75 bladder calculi patients, fifteen were below 18 years, thirty four were between 19 to 60 years, and twenty six were between 61 to 90 years. Out of 75 patients of bladder calculi, 17 patients had Benign Prostatic Hyperplasia for which Transurethral Resection of Prostate and Cystolithotripsy was performed. Six Patients had Stricture Urethra for which Visual Internal Urethrotomy and Cystolithotripsy was performed. Rest 52 Patients Underwent Cystolithotripsy . Among 75 patients of bladder calculi, 49 patients had component of Calcium Oxalate Monohydrate(COM). **Conclusion-** Raman and IR spectroscopy are reliable techniques for identifying mineral constituents in Bladder calculi. Calcium Oxalate Monohydrate is the most common calculus followed by uric acid.

KEYWORDS : Urolithiasis, Bladder calculi, Fourier Transform Infrared Spectroscopy, Raman Spectroscopy, Stone Analysis

INTRODUCTION

Bladder stones are the most common manifestations of lower urinary tract stones, currently accounting for 5% of all urinary stone disease and approximately 1.5 % of urological Hospital admissions in industrial Western Nations. [1]

Primary calculus are those which develop in the absence of any known functional, anatomic, or infectious factors and the term does not necessarily imply that Stones have formed de novo in the bladder. They are most common in children less than 10 year of age, with a peak incidence at 2 to 4 years of age. [2] The disease is more common in boys than in girls, with ratios ranging from 9:1 to as high as 33:1 in India. This is attributed to predominant cereal-based diet that is poor in animal protein and low in phosphate. Lower dietary intake of phosphate not only leads to hypophosphaturia but also to hyperammonuria, promoting the precipitation of both calcium oxalate and ammonium acid urate.

Secondary bladder calculi are typically found in man older than a 60 and are usually in seen with lower urinary tract obstruction, which prevents complete bladder emptying. [3] Bladder Outlet obstruction resulting in incomplete emptying and the retention of stone fragments is the most common predisposing factor for bladder stone formation in non neurogenic bladder and is present in 45% to 79% of all patients diagnosed with Vesical calculi. [4] In men, outlet obstruction is generally related to benign prostatic hyperplasia, whereas urethral kinking from a cystocele or pelvic organ prolapse is often the culprit in females. Uretheral stricture, bladder neck contracture, and bladder diverticula are also secondary causes that my interrupt normal voiding patterns.

The composition of stones resulting from anatomic obstruction varies with region and race. In Europe, Struvite, Calcium Phosphate, and Uric Acid predominant, where as in Japan uric acid calculi are uncommon and Calcium stones are increasing in incidence, now presenting 72% of all stones found in recent series. Calcium oxalate comprises the majority of bladder stone found in United States, although uric acid stones predominant among the American Jewish population. [5]

Although Fourier Transform Infrared Spectroscopy (FTIR) analysis has been extensively utilized to analyze renal calculi, it has rarely been utilized to analyze bladder calculi. FTIR can detect both crystalline and amorphous materials. The Raman technique is strongly complementary to FTIR as sample preparation is not required. Principles of both Raman and FTIR are same as both provide information on the characteristic vibrational frequencies and structural aspects of chemical function groups. In FTIR, Infrared radiation is absorbed by the vibrating chemical functional group that make up a sample, producing characteristic absorption bands which facilitate identification of those groups. While Raman technique is based on scattering of light radiation by the chemical function groups and produces characteristic Raman brands, which helps in identification of functional group as in FTIR, it is more structure sensitive with the ability to differentiate spectral aspects. In the present paper we discuss spectroscopic analysis of bladder calculi by FTIR and Raman spectroscopy to determine their minerological content. FTIR is the primary, most commonly done analysis. In conjunction with FTIR, IR Raman spectroscopy was used to analyze the efficiency of FTIR.

Powder X-Ray diffraction (PXRD), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), Total reflection X-Ray Fluorescence Spectroscopy (TXRF) and Infrared (IR) Imaging techniques has provided additional support for the proposed foolproof identification of the mineral constituents. Powder X-Ray diffraction is the most suitable method to study solids with multiple components. Thermogravimetric Analysis can predict major and minor components of the samples, and used for qualitative and quantitative analysis of stone samples. The diversity in the lithogenesis process significantly influences the composition, stone morphology and internal structure. The pattern in which minerals are distributed in the stone matrix is defined by the mineral accumulation process during the crystal growth and can be analysed using Scanning Electron Microscopy. Major and minor components of the stones are analysed using IR. The location of each component is related to the pathological conditions at the time of mineralization. The spatial distribution of the components can be linked to the metabolic changes that occur during stone formation. Using Total reflection X-Ray Fluorescence Spectroscopy the Identification of the elements in the stone samples provides a complete quantitative elemental analysis of the samples.

MATERIALS AND METHODS

75 Urinary Bladder Stones were collected post lithotripsy washed in distilled water, dried and used. The spectra (4000–400 cm^{-1}) were measured on ThermoFisher NICOLET-6700 FT-IR spectrometer which consisted of Alum standard ETC. Ever-Glo IR source and DTGS detector equipped with KBr windows. The surfaces of stone samples were cut with a sterile scalpel and then ground with agate mortar and pestle. A pellet was prepared for each sample with a dry KBr in the ratio 1:100. The pellet obtained was transferred to the instrument to obtain spectra with a resolution of 4 cm^{-1} at 100 scans. The Raman spectra were measured on a ThermoFisher NXR FTRaman Module, with a resolution of 4 cm^{-1}. This module consisted of Nd:YVO4 laser (1064 nm) as an excitation source. The interferometer bench comprised a CaF2 beam splitter and LN2 cooled Ge detector. After IR and Raman spectroscopy, marker bands of all the minerals were verified with OMNIC library spectrum database. The uric acid (UA) as an organic molecule is amenable to ab initio quantum chemical calculations and therefore we have computed molecular geometry and IR and Raman spectra at density functional theory's B3LYP/6-31++G level. These calculations were performed using Gaussian 09 W and GaussView 5.0.9 suite of programs.

RESULTS

Among 75 patients of bladder calculi, fifteen patients were below the age of 18 years, thirty four patients were between 19 to 60 years, and twenty six patients were between 61 to 90 years. (figure 1)

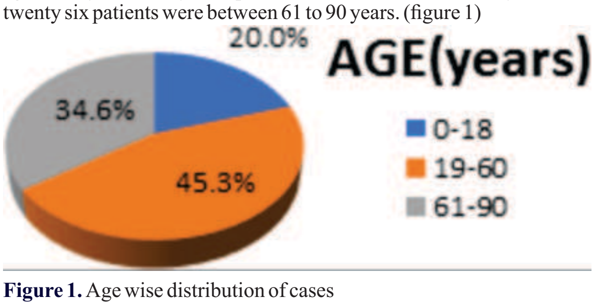

Figure 1. Age wise distribution of cases

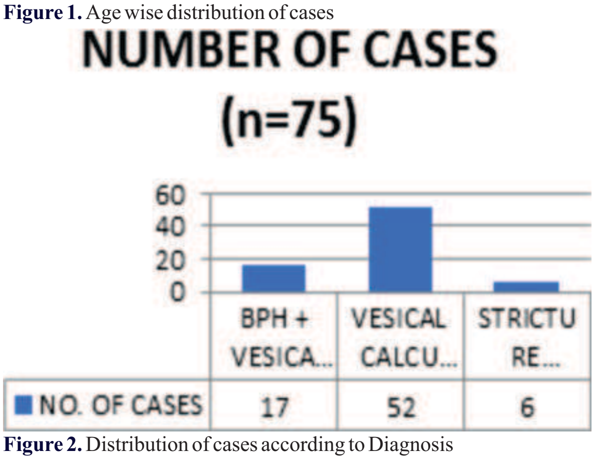

Figure 2. Distribution of cases according to Diagnosis

Out of 75 patients of bladder calculi, 17 patients (n=75) had Benign Prostatic Hyperplasia for which Transurethral Resection of Prostate and Cystolithotripsy was performed. Six Patients (n=75) had Stricture Urethra for which Visual Internal Urethrotomy and Cystolithotripsy was performed. Rest 52 Patients (n=75) Underwent Cystolithotripsy. (figure 2). Among 75 patients of bladder calculi, 49 (n=75) patients had component of Calcium Oxalate Monohydrate(COM), of which only five had pure Calcium Oxalate Monohydrate composition, and rest 44 samples detected to have mixed mineralogical composition.

Among fifteen patients below 18 years of age, the mineral composition is given in table 2. In the present study maximum (10/18) Pediatric bladder stones had component of Calcium Oxalate Monohydrate (COM). These proves that maximum stones where migratory stones from kidney.

Among Twenty three patients who had vesical stone with bladder outlet either BPH or Stricture, sixteen patients had component of Calcium Oxalate Monohydrate(COM). We selected two samples at random in order to cross verify both the IR and the Raman spectrum analysis by various analytical methods on a notional but conclusive basis. The analytical methods used are total reflectance X-ray flouresence spectroscopy for elemental analysis, powder X-ray diffraction and thermogravimetric analysis for component analysis, scanning electron microscopy for morphological features with correlation to components, infrared microscopy for chemical imaging, and component analysis. MAPH + COD for (BS39) and COM + COD for (BS9).

Table 1. Distribution of cases according to Mineral composition, Calcium Oxalate Monohydrate (COM, whewellite), Calcium Oxalate Dihydrate (COD, weddellite), Magnesium Ammonium Phosphate Hexahydrate (MAPH, struvite), Calcium Hydrogen Phosphate Dihydrate (CHPD, brushite), Pentacalcium Hydroxy Triphosphate (PCHT, hydroxyapatite) Ammonium Urate (AU), Uric Acid Dihydrate (UAD) and Uric Acid (UA)

Identified Minerals as Single /Combinations	Number of Bladder stones	Identified Minerals as Single/Combinations	Number of Bladder stones
COM	5	PCHT, WHITLOCKITE	1
COM, COD	24	COM, PCHT	1
COM, UA	1	COM, COD, PCHT	2
COM, COD, UAD	5	MAPH, COD	2
COM, COD, UA	8	MAPH, UAD	1
UA, UAD	10	COM, COD, CHPD	3
AU	11	PCHT, MAPH, CHPD	1

Table 2. Distribution of cases according to Mineral composition in Pediatric age group, Calcium Oxalate Monohydrate (COM, whewellite), Calcium Oxalate Dihydrate (COD, weddellite), Ammonium Urate (AU), Uric Acid Dihydrate (UAD) and Uric Acid (UA)

INERALS IDENTIFIED	NUMBER OF STONE SAMPLES
COM, COD	3
COM	1
COM, COD, UA	6
AU	2
UA, UAD	3

Two samples that were subjected to XRD, TGA, SEM, TXRF, and IR microscopy analyses show that the outcomes are in line with the findings of the IR and Raman spectrum analyses.Furthermore, it has been shown that a fusion of Raman and IR analysis is the most accurate method for identifying the mineral components.

DISCUSSION

Raman process spectroscopy is based on the study of scattered light, while IR spectroscopy is based on the absorption of the light. Raman spectroscopy provides information about intramolecular and intermolecular vibrations and helps to get a more complete data of the reaction. While the FTIR method places restrictions on sample thickness, homogeneity, and dilution to prevent saturation, the main benefit of Raman spectroscopy is that it requires little to no sample preparation. The main benefit of FTIR over the other is how they handle interference differently. Fluorescence may interfere with the ability of taking Raman spectra, although this would not affect FTIR. Both techniques provide same vibrational properties, supplementing & -complementing each other. In both cases, vibrational modes with frequencies due to molecular functional groups are characteristic and hence can be used to determine the presence of the groups and intensities.

Table 3. Identified mineral constituents as major/minor components in five stone samples by IR & Raman spectroscopy, verified by Powder X-ray Diffraction (PXRD), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), IR Microscopy and Total X-ray Fluorescence techniques & compared with OMNIC Database, Calcium Oxalate Monohydrate (COM), Calcium Oxalate Dihydrate (COD), Magnesium Ammonium Phosphate Hexahydrate (MAPH)

Technique Samples	IR	Raman	OMNIC	PXRD	TGA	SEM	IR Microscopy	TXRF
BS 39	MAPH (major)+ COD (minor)	COD + MAPH	MAPH(80%)+ COD(10%)	MAPH+ COD	MAPH (61%)	Amorphous nature	Random distribution	P(92%),Cl,,K,Ca, Mn, Fe,Ni,Cu, Zn, As,Br,Kr,Rb,Cs
BS 9	COM (major)+ COD (minor)	COM + COD	COM(65%)+ COD(35%)	COM+ COD	COM (56%)	COD crystals on COM	Random distribution	Ca(84%),Fe,Cu,Zn,As, Br K,Ti, Cr,Mn,Sr,Zr,Mo.

Bijapur district is one of the stone belt regions of Karnataka. An approximate understanding of the etiology will be provided by stone analysis, which will aid in the development of preventative measures. In the present study among 75 patients of bladder calculi, 49(65.3%) patients had component of Calcium Oxalate Monohydrate(COM), of which only five had pure Calcium Oxalate Monohydrate composition, and rest 44 samples detected to have mixed mineralogical composition. Bhat et al. [6] Conducted FTIR analysis of 122 Bladder stones in Rajasthan a stone belt region of India. Calcium oxalate was the most frequent composition (76/122 (62.3%). It was present in 6/18 (33.3%) in 8-12 years of age group, 10/20(50%) in 13-18 years of age group and 60/84(71.4%) in more than 18 years. Calcium oxalate was the most frequent composition in all age group except 8 to 12 years, while rest were composed of varying amount of struvite, calcium phosphate, and uric acid.

Zhang D et al. [7] did a FTIR analysis of 42 bladder calculi, following are the various compositions of the bladder calculi, 21 patients had calcium oxalate, six had calcium phosphate, eight patients had struvite, 5 had uric acid and two patients had cysteine. Calcium oxalate was the most frequent composition. Imran K et al. [8] did a FTIR analysis of 101 bladder calculi, Calcium oxalate was the most frequent composition 69(68%). AAU stones were more prevalent in children 0–5 years (73%) as compared to children 11–15 years (60%). CaOx stones were more prevalent in children 11–15 years (90%) as compared to children 0–5 years (62%). Similar were the findings in pure stones with CaOX in older children and AU in children(<5 years). The number of patients in our study are small and a larger study may be necessary to get the true picture of the stone composition in this belt. We did not stratify our results on the basis of boby mass index, residence of the patient, the occupation of the patient, dietary habits, socio-economic status, which can have implications upon the stone composition.

CONCLUSION

FTIR spectroscopy and Raman spectroscopy are reliable techniques for the identification of mineral constituents in Bladder calculi. Calcium Oxalate Monohydrate(COM) is the most common calculus detected in North Karnataka Population. We recommend that stone analysis should routinely be conducted in all cases. This would help in the prevention of stone recurrence.

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