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Analysis of Kidney and Gall Stones of Patients from North-East Bangladesh by FTIR

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Abstract

Kidney and gallstones represent a prevalent and costly health problem. This study aimed to define patterns of stones and identify chemical compositions in the sample of symptomatic kidney and gallstones from North-East part of Bangladesh (Sylhet City, Sylhet) using infrared spectroscopy. Kidney and gallstones were recovered from patients of Osmani Medical College and Hospital, Sylhet, Bangladesh. A total of 5 kidney and 3 gall stone samples were collected randomly and analyzed for composition by Fourier Transform Infrared Spectroscopy (FTIR). Among the five kidney stone samples, KDS 01 was mixture of calcium oxalate monohydrate and calcium phosphate, KDS 02 and 03 were calcium oxalate and uric acid stones, KDS 04 was uric acid stone and KDS 05 was calcium oxalate dehydrate and calcium phosphate. Among the three gall stone samples, GBS 01 was cholesterol stone, GBS 02 was mixed stone and GBS 03 was pigment stone.

Keywords: Kidney stones, Gall stones; FTIR; Calcium oxalate stones; Cholesterol stones.

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1. Introduction

Worldwide, urolithiasis is the third most common urological diseases affecting both men and women. Both genetic and environmental factors contribute to stone formation. Urolithiasis is a global problem affecting human beings for several centuries. The annual incidence of urolithiasis in the western world is 0.5% [1-3]. Most of the urinary stone samples comprised mineral crystals aggregated into random clumps of varying sizes that

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are formed within the kidney in a relatively open environment by processes not orchestrated by specialized cellular or macromolecular machinery [4]. Recent epidemiological studies have suggested an increased frequency of kidney stone disease in all age groups during the last decades [5]**.** Chemical composition of gall stones is essential for aetiopathogensis of gallstone disease. Studies have shown that dietary intake of total calories in the form of carbohydrates and fats are associated with high triglyceride levels in gallstone patients [7]. Chemical constituents of the majority of human gallstone are cholesterol and pigment stones [8]. Other substances found in gallstones include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals [9, 10]. Cholelithiasis or gallbladder stones are one of the major surgical problems in many hospital admission and surgical interventions [6, 7]. Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world [11**,** 12]. Clinically, high risk groups with small gallstones have recently been defined [13]. Significant progress has been made both in the genetics of gallstone formation [14, 15] and in the molecular biology of bile excretion [16-18]. In Bangladesh, recent years has seen an increasing trend in the number of gallstone cases.

FTIR spectroscopy is an effective method for the evaluation of kidney and gallstones composition [19]. We have chosen FTIR as a tool for identifying stones because of easy availability of this machine in our country compared to SEM, TEM, XRD etc. Qualitative and quantitative analyses were performed using the suitable chemical methods and titration techniques.

2. Experimental

2.1. *Materials and methods*

The fresh stones were collected from patients of Osmani Medical College and Hospital, Sylhet, Bangladesh during February – September, 2009 and the stones were kept at 4ºC until further analysis. The age of patients ranged from 25 to 83 years, including men and women. The stones were washed with distill water for an hour to remove waste and bile and dried at 60ºC for 4 h. During analysis the kidney stones were crushed by mortar pastel and gall stones were cut into two halves by using a surgical blade. Portion of stones were ground with a pestle and mortar, 2 mg of sample was mixed with 40 mg of spectral grade KBr crystals, and grounded as fine as possible to make the 13 mm diameter disc. The FTIR measurements were performed using a Perkin Elmer Spectrum RX1 Model FTIR spectrometer in the frequency range 4000–400 cm^{-1} at 4 cm^{-1} resolution. To obtain a high signal/noise ratio 100 scans were accumulated for each sample.

The presence of the cations and anions were conformed using the qualitative analysis [20]. Metal ions and phosphate in the renal stones were studied after a standard digestion process using concentrated HNO_3 at 250°C for at least 30 min. However, prior to determining presence of phosphate, digested solutions were first neutralized using 1.0 M NaOH. 1.5 mL of ascorbic acid solution (7% w/v) followed by 1.5 mL of mixed reagent

(45 mL of molybdate dissolved in 200 mL of conc. H_2SO_4 followed by 5 mL of tartrate solution) was added for color development. Absorbance of the resulting colored solution was then recorded at a wavelength of 882 nm [21]. Iron was determined using spectrophotometric technique [22] and the quantity of calcium was determined using the quantitative chemical analysis [23]. Magnesium was determined by EDTA titration.

3. Results and Discussions

The chemical components of kidney and gall stones samples KDS (1, 2, 3, 4, and 5) and GBS (1, 2, and 3) and its corresponding IR absorption band of kidney and gallstones are characteristically and systematically assigned in Tables 1- 4.

3.1. *Biochemical analysis of kidney and gallstones*

The stones were collected from Osmani Medical College and Hospital, Sylhet, Bangladesh and washed with distilled water to remove loose debris. For analyzing stone constituents, stones were hack-sawed into halves and the powder obtained from all layers was intimately mixed in agate mortar. This powder stone sample was used for the analysis.

A total of five kidney and three gall stones were collected and the kidney samples were coded as KDS 01, KDS 02, KDS 03, KDS 04, and KDS 05, and the gall stones were coded as GBS 01, GBS 02 and GBS 03. From the biochemical analysis, the sample KDS (1, 2, 3, 4, 5) and GBS (1, 2, 3) shows the presence of the following constituents and their quantitative analysis are shown in Tables 1 and 2.

Table 1. Qualitative analysis of kidney and gall stones of a region (Sylhet City) of Bangladesh.

Samples	Iron	Calcium	Magnesium	Copper	Chloride	Inorganic phosphate	Oxalate
KDS 01	Present	Present	Present	Absent	Absent	Present	Present
KDS 02	Do	Do	Do	Do	Do	Do	Do
KDS 03	Do	Do	Do	Do	Do	Do	Do
KDS 04	Do	Do	Do	Do	Present	Do	Do
KDS 05	Do	Do	Do	Do	Absent	Do	Do
GBS-01	Do	Do	Do	Do	Present	Do	Do
GBS-02	Do	Do	Do	Do	Do	Do	Absent
GBS-03	Do	Do	Do	Do	Do	Do	Do

3.2.1. *FTIR spectroscopy of kidney stone sample 1*

The sample 1 (KDS01) was found to be mixture of calcium oxalate monohydrate (COM) and calcium phosphate. This was characterized from the most important bands at 3488

cm^{-1}, 3258 cm^{-1} and 3062 cm^{-1} symmetric and asymmetric H-O-H stretch. Absorption at 1640 cm^{-1} and 1320 cm^{-1} may be due to O-C=O symmetric and asymmetric stretching respectively. The bands at 661 cm^{-1} and 781 cm^{-1} are due to the out of plane of O-H bending and C-H bending mode respectively and band at 519 cm^{-1} arises due to O-C-O in plane bending. The absorbance values of calculi containing COM and calcium phosphate with their wave numbers are shown in Table 3.

Table 2. Quantitative analysis of stones of a region (Sylhet City) of Bangladesh.

Types of stone	pH	Conductivity (µs/cm)	Turbidity (NTU)	Alkalinity (mgl^{-1})	Fe^{2+} (mg/g)	PO_4^{3-} (mg/g)	Mg^{2+} (mg/g)	Ca^{2+} (mg/g)
KDS-01	8.2	340	9.02	238	0.45	0.05	2.73	6.17
KDS-02	7.6	408	13.95	319	0.38	0.09	3.11	7.09
KDS-03	8	378	8.48	283	0.32	0.12	2.28	8.77
KDS-04	8.1	352	14.29	407	0.42	0.71	4.10	7.25
KDS-05	7.9	230	9.61	355	0.37	0.15	3.43	6.89
GBS-01	5.8	480	8.67	223	0.21	3.99	4.52	3.63
GBS-02	6.9	398	13.98	261	0.16	5.12	5.06	3.86
GBS -03	6.4	411	10.63	248	0.18	4.86	6.40	4.16

3.2.2. *FTIR spectroscopy of kidney stone sample 2 and 3*

The samples 2 (KDS02) and 3 (KDS03) were found to be mixture of calcium oxalate and uric acid stone. These bands (1608 cm^{-1}, 1613 cm^{-1}) and (1321 cm^{-1}, 1318 cm^{-1}) are due to the O-C=O symmetric and asymmetrical stretching respectively. The band at (1641 cm^{-1}, 1638 cm^{-1}) are assigned to C=O stretching. The bands at 1033 cm^{-1}, and (781 cm^{-1}, 787 cm^{-1}) are due to the N-H stretching, C-H bending or C-N stretching of aromatic or N-H out of plane and in plane bending respectively. The bands at (604 cm^{-1}, 606 cm^{-1}), 587 cm^{-1} and 516 cm^{-1} respectively are from ring breathing mode, skeletal ring deformation and O-C-O in plane bending (Table 3).

3.2.3. *FTIR spectroscopy of kidney stone sample 4*

The spectra of kidney stones sample 4 (KDS04) show characteristic IR band of uric acids. The absorbance from 3685 cm^{-1} to 2293 cm^{-1} are due to the heteroaromatic stretching. The bands at 1670 cm^{-1}, 1582 cm^{-1} and 1312 cm^{-1} are respectively from C=O and C=N stretch and O-H deformation. The peaks at 781 cm^{-1} and 521 cm^{-1} show the N-H out of plane and inplane bending and skeletal ring deformation (Table 3).

Table 3. Type, occurrence, and IR bands of principal components observed in kidney stones.

Samples	Types of stones	Principle IR-bands observed in present study	Assignments
Sample 01(KDS01)	Mixture of calcium oxalate monohydrate and calcium phosphate	3488 cm^{-1}, 3258 cm^{-1} and 3062 cm^{-1}	Symmetric and asymmetric H-O-H stretch
		1640 cm^{-1}	O-C=O asymmetric stretching
		1320 cm^{-1}	C-C symmetric stretching
		1050 cm^{-1}	Presence of PO_4^{3-} group
		781 cm^{-1}	C=O asymmetric stretching
		661 cm^{-1}	Out of plane of O-H bending
		519 cm^{-1}	O-C-O in-plane bending.
Sample 02 (KDS02) and Sample 03 (KDS03)	Mixture of calcium oxalate and uric acid stone	3477 cm^{-1}	Symmetric and asymmetric H-O-H stretch
		1641 cm^{-1}, 1638 cm^{-1}	C=O stretching
		1608 cm^{-1}, 1613 cm^{-1}	O-C=O symmetric stretching
		1321 cm^{-1}, 1318 cm^{-1}	O-C=O asymmetric stretching
		1033 cm^{-1}	N-H stretching or ring vibration
		781 cm^{-1}, 787 cm^{-1}	C-H bending or C-N stretching of aromatic or N-H out of plane and in plane bending
		604 cm^{-1}, 606 cm^{-1}	Ring breathing mode
		587 cm^{-1}	Skeletal ring deformation
		516 cm^{-1}	O-C-O in plane bending
Sample 04 (KDS04)	Uric acid stones	3685 cm^{-1} to 2293 cm^{-1}	Heteroaromatic N-H stretching
		1670 cm^{-1}	C=O stretch
		1582 cm^{-1}	C=N stretch
		1312 cm^{-1}	O-H deformation
		781 cm^{-1}	N-H out of plane and in-plane bending
		521 cm^{-1}	Skeletal ring deformation.

Table 3 (*contd.*)

Sample 05 (KDS05)	Calcium oxalate dehydrate and calcium phosphate	1616 cm^{-1}	C=O asymmetrical stretching
		1317 cm^{-1}	C-O symmetric stretch.
		1466 cm^{-1}	Presence of $–NH_2$ group
		1053 cm^{-1}	Presence of PO_4^{3-} group
		782 cm^{-1}	C-H bending
		516 cm^{-1}	O-C-O in plane bending

3.2.4. *FTIR spectroscopy of kidney stone sample 5*

Sample 5 (KDS05) shows the following stretching mode vibrations. The bands at 1616 cm^{-1} and 1317 cm^{-1} are respectively due to the C=O and C-O stretching vibrations. The vibration at 1466 cm^{-1} shows the presence of $–NH_2$ group and the band at 1053 cm^{-1} shows the presence of PO_4^{3-} group. The bands at 782 cm^{-1} and 516 cm^{-1} are due to the C-H bending. The absorbance at 3478 cm^{-1}, 782 cm^{-1} and 516 cm^{-1} may be due to COD (calcium oxalate dehydrate). Hence the sample 5 analyzed by FTIR might be a mixture of COD and calcium phosphate (Table 3).

3.3.1. *FTIR spectroscopy of GBS 01*

Gallstones sample 1 is characterized by the band at 3441 cm^{-1} due to the large CH_2 asymmetric stretching vibration, CH_3 asymmetric stretching at 2939 cm^{-1}, CH_2 bending at 1458 cm^{-1} and a sharp peak at 1049 cm^{-1} can be attributable to ring deformation of cholesterol [24] (Table 4).

3.3.2. *FTIR spectroscopy of GBS 02*

The spectra of gallstones sample 2 show characteristic IR band of mixed gallstones. The bands at 3319 cm^{-1}, and 2930 cm^{-1}, arise due to the CH_2 asymmetric stretching, CH_3 asymmetric stretching. The presence of specific peak at 2931 cm^{-1} is observed in all mixed stones. The band at around 2355 cm^{-1} corresponded to N-H and C-H stretching. The existence of amides I and II is confirmed by peaks at 1666 cm^{-1} and 1556 cm^{-1}. The calcium bilirubinate has characteristic bands at 1249 cm^{-1} (amide III), which is assigned to (C-O) stretching or C-N stretching coupled with N-H deformation (C-N) [25, 26]. The doublets at 1373 cm^{-1} are attributed to the (CH_3) bending vibration of cholesterol in the mixed gallstone [25, 27, 28]. The peaks at 1458 cm^{-1} and 956 cm^{-1} indicated the presence of CH_2 bending and calcium phosphate (Table 4) [29].

3.3.3. *FTIR spectroscopy of GBS 03*

The spectra of gallstone sample 3 shows characteristic IR band of pigment stone. The bands at 3319 cm^{-1}, and 2930 cm^{-1}, are due to the CH_2 and CH_3 asymmetric stretching. The spectra of gallstones show characteristic IR bands for blirubin in the region between 1500- 1700 cm^{-1} and the triplet in that region which is an indication of the pigment stones [30, 31]. The IR peaks at 1666 cm^{-1}, 1627 cm^{-1} and 1543 cm^{-1} are C=O asymmetric stretching weakly coupled with C-N stretching and in plane N-H bending (amide I), C=O carboxylate group and amide II, respectively (Table 4).

Table 4. Type, occurrence, and IR bands of principal components observed in gall stones.

Samples	Types of stones	Principle IR-bands observed in present study	Assignments
GBS 01	Cholesterol	3441 cm^{-1}	CH_2 asymmetric stretching
		2939 cm^{-1}	CH_3 asymmetric stretching
		1458 cm^{-1}	CH_2 bending
		1049 cm^{-1}	ring deformation of cholesterol
GBS 02	Mixed	3319 cm^{-1}	CH_2 asymmetric stretching
		2930 cm^{-1}	CH_3 asymmetric stretching
		2355 cm^{-1}	N-H and C-H streching
		1666 cm^{-1}	C=O asymmetric stretching weakly coupled with C-N stretching and in plane N-H bending (amide I)
		1556 cm^{-1} and 1249 cm^{-1}	Amide II and amide III respectively
		1373 cm^{-1}	bending vibration of cholesterol
		1458 cm^{-1}	CH_2 bending
		956 cm^{-1}	P=O symmetric stretching
GBS 03	Pigment	3319 cm^{-1}	CH_2 asymmetric stretching
		2930 cm^{-1}	CH_3 asymmetric stretching
		1666 cm^{-1} and 1627 cm^{-1}	C=O asymmetric stretching weakly coupled with C-N stretching and in plane N-H bending (amide I) and C=O carboxyl ate group, respectively
		1543 cm^{-1}	Amide II

4. Conclusion

Identification of the constituents of kidney and gallstone is the first step in medical diagnosis. Spectroscopy is one of the major analytical tools for analyzing the chemical composition of kidney and gallstones qualitatively as well as quantitatively. An FTIR spectrum was found to be very sensitive, reliable and less time consuming technique for classifying the human kidney and gallstones. The study showed that calcium oxalate type kidney and cholesterol type gall stones are common in patients of Sylhet and adjoining areas, may be due to having excess oxalate rich (e.g. tea) and cholesterol rich (e.g. beef) foods.

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