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MINERALOGICAL COMPOSITION AND STRUCTURAL CHARACTERIZATION OF KIDNEY STONE PATIENTS AT A SINGLE TERTIARY CARE CENTRE IN SOUTH INDIA

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Abstract

Purpose: Urinary lithiasis is globally prevalent irrespective of gender and age. Now, it is a vital step to find out the mineral composition of kidney stones along with the ins and outs of this disease to develop prophylactic measures. The present work represents identifying minerals and their composition of 30 renal calculi collected from patients suffering with kidney stones formation with the aid of infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM).

Patients & Methods: Prominent pioneering analytical techniques were employed to 30 renal calculi sample collected through nephrectomy or percutaneous nephrolithotomy surgical procedures. They were studied for biochemical and spectroscopic analysis. All the 30 sampleswere investigated for ammonia, carbonate, oxalate, calcium and phosphate, uric acid, cystine, magnesium followed by FT-IR, XRD and SEM analysis.

Results: In this study, incidences were more amongst men with an age between 40-50 years, preferably non-vegetarians. The results presented existence of ammonia, oxalate and cystine in most of the renal calculi samples. The cutting-edge tools revealed the presence of struvite, whewellite, weddellite with phosphate blend and magnesium ammonium phosphate.

Conclusion: The results of the current investigation displayed the diverse chemical compositions of urinary stones. The results of FT-IR, XRD, and SEM are not only helpful in identifying the different mineral forms of urinary stones but also helps customised medicationfor the patients.

Keywords: Urolithiasis, Renal Calculi, FT-IR Analysis, X-ray Diffraction, Scanning ElectronMicroscopy.

Introduction

Urolithiasis (nephrolithiasis or kidney stone disease) is an occurrence of one or more stones (kidney stones or urinary/renal calculi) in the human urinary tract (kidney, ureter, and bladder), which is usually an unusual deposition of minerals and salts in various forms and dimensions.[1] Though, worldwide incidences estimated from 1% to 13%,[2] India, China and Russian Federation share nearly half the burden of this disease.[3] In India, occurrence of urolithiasis is 7.9%,[4] amongst which there are reports of renal damage and loss of kidney function.[5] The etiology of urolithiasis is multifactorial; gender, age, ethnic, racial, dietary and lifestyle factors, environmental, metabolic disorders, and urinary tract infections are relatively common.[6,7] The formation of polycrystalline renal calculi initiates when instability between precipitation and solubility of urinary volume and concentrations of minerals and salts in the urinary system occurs, later aggregating enough in size so cannot be excreted naturally and hence continue to gather in kidney, ureter, or bladder.[8] Based on their mineralogical composition and pathogenesis, renal calculi are classified into four types: calcium oxalates (80%), struvite (10-15%), urate (3-10%) and cysteine (<2%).[9] Calcium oxalate monohydrate (whewellite, $CaC_2O_4.H_2O$) and Calcium oxalate dihydrate (weddellite, $CaC_2O_4.2H_2O$) are most frequently occurring renal calculi,[10,11] and also found in repeatedly with phosphate blend *viz.*, brushite ($CaHPO_4.{}_2H_2O$), calcium phosphate ($Ca_{10}(PO_4)_6.2H_2O$) followed by dolomite ($CaMg(CO_3)_2$), gypsum ($CaSO_4.2H_2O$) and calcium carbonate ($CaCO_3$).[11,12] Among the patients with severe urinary tract infection struvite calculi *i.e.,* magnesium ammonium phosphate ($MgNH_4.PO_4.6H_2O$) are more prevalent and encompassing pathogenic microbes.[9,12] The hyperuricosuria patients associated with elevated purine ingestion, dehydration and gout disease, owns urate calculi ($C_5H_4N_4O_3$) and occasionally reported cystine calculi are linked with rBAT gene on chromosome-2 which is an autosomal recessive disorder leading to cysteine amino acid leakage into urine due to decreased renal tubular absorption.[13] The urolithiasis

patients are associated with severe metabolic syndromes, diabetes, hypertension, cardiovascular diseases and obesity followed by chronic kidney disease leading to end-stage renal disease.[14] Though there are plenty of treatment options available, it is essential to understand the structural and morphological depiction, phase composition and formation process which may help in prohibiting the recurrence of renal calculi by adapting the suitable diet and other risk factors. Innumerable cutting-edge analytical techniques *viz.,* Fourier Transform Infrared spectroscopy (FT-IR) to comprehend mineralogical composition and chemical functional groups; X-ray diffraction (XRD) to illustrate and categorize crystalline compounds and scanning electron microscopy (SEM) coupled with energy dispersive X-rays (EDX) for surface morphology, qualitative and quantitative classification of renal calculi.[15] Currently, with the lack of qualitative and quantitative data of renal calculi amongst the patients from Northern parts of Karnataka our study aims for mineralogical composition and structural elucidation of renal calculi with the aid of FT-IR, XRD and SEM followed by finding an association between age and sex and the prevalence of renal calculi in view of their mineral composition.

Materials and Methods

Sample Collection

A total of 30 renal calculi were collected from patients through nephrectomy or percutaneous nephrolithotomy surgical procedures, who were admitted to a tertiary care centre in South India. These renal calculi were washed with double distilled water, later with surgical spirit to remove any blood clots or mucus. Subsequently, they were dried in an oven at 60° C and 100 mg was crushed to fine powder with mortar and pestle. All patients were provided with written informed consent and the study was permitted by ethical committee (MDC/DoME/60, Dated: 24/05/2019).

Biochemical Analysis

Test for Ammonia

0.5 mg of renal calculi powder along with 20% NaOH solution was taken in a test tube and was heated slightly. A moist red litmus paper was introduced in the solution and observed until red litmus paper turns blue.

Test for Carbonate

A 0.5 mg of renal calculi powder was taken and slowly alongside of the test tube 1N sulphuric acid was added. The presence or absence of effervescence was observed.

Test for Oxalate

A 0.5 mg of renal calculi powder was taken in a test tube along with a more amount of dilute sulphuric acid and heated slightly. Later, dilute potassium permanganate solution was added slowly. The presence or absence of decolorization of the $KMnO_4$ solution was observed.

Test for Calcium and Phosphate

A 0.5 mg of renal calculi powder was taken in a test tube along with 1ml of concentrated nitric acid, mixed thoroughly and divided into two portions. To the 1st portion, 1ml of ammonium molybdate solution was added and heated. The presence or absence of appearance of a yellow precipitate is observed. To the 2nd portion, 1ml of distilled water was added. The solution was made alkaline by adding ammonia and a small amount of 5% potassium oxalate solution was added. The presence or absence of silky white precipitate is observed.

Test for Uric Acid

A 0.5 mg of renal calculi powder was taken in a test tube along with 14% sodium carbonate solution and mixed thoroughly. Then few drops of Benedict's uric acid were added. The presence or absence of blue colour is observed.

Test for Cystine

A 0.5 mg of renal calculi powder was taken in a test tube and few drops of concentrated ammonium hydroxide solution was added until it gets dissolved. The presence or absence of complete evaporation is observed.

Test for Magnesium

A 0.5 mg of renal calculi powder was taken in a test tube along with dilute HCl was added. The solution was boiled, cooled, and filtered. To the filtrate 1ml of diluted ammonium hydroxide and 1ml of 4% ammonium oxalate was added and filtered again. To this filtrate 1ml of potassium phosphate and 1ml of dilute ammonium hydroxide was added. The presence or absence of white precipitate is observed.

Spectroscopic Analysis

FTIR analysis

FT-IR analysis was performed with a SHIMADZU-IR-Affinity-1 instrument and the spectra were taken between 400-4000cm^{-1} range using IR grade KBr as standard. One part of powder sample was triturated with ninety-nine parts of KBr and kept in a sample bucket. The found spectra were analysed for their standard characteristic peaks.

XRD analysis

The XRD analysis of powdered samples were characterized using SmartLab SE, Rigaku Corporation instrument. The diffraction patterns were recorded using Cukbeta source and within the angular range (2θ): 5° to 140° and hence the crystalline phases of renal stone samples were identified.

Scanning Electron Microscopy (SEM) - Energy Dispersive X-rays (EDX)

The SEM-EDX was carried out using a JSM-IT500 InTouchScope^{TM} equipped with Energy Dispersive X-ray Spectrometer. An accelerating voltage of 5kV was used.

Results

Based on the information gathered, patients were categorized into four groups considering their age *i.e.,* Group-I to IV. The age (in years) between 11 to 20 as Group-I, 21 to 30 as Group-II, 31 to 40 as Group-III and 41 to 50 as Group-IV. Amongst these, Group-I had 6.6% (n=2), Group-II had 26.6% (n=8), Group-III had 23.3% (n=7) and Group-IV had 43.5% (n=13) number of patients, in which patients who prefer non-vegetarian food were 20 (66.7%) and vegetarian were 10 (33.3%). The outcome of biochemical analysis showed the presence of

ammonia, oxalate and cystine in most of the renal calculi samples (sample 1 to 30), whereas absence of calcium and potassium within the same samples (sample 2, 4, 9, 11, 13, 14, 17, 19, 20, 21, 22, 24, 27, 29) was also substantial. It was also evident that, presence of carbonate (sample 3, 5, 8, 22, 23), uric acid (sample 1 to 7, 10, 17, 28) and magnesium (sample 3, 4, 5, 10, 12, 13, 15, 16, 18, 23, 24, 26 to 29) was least **(Table 1)**.

FTIR, XRD and SEM

The samples, SNV1, SNV3, SV1, SV3 and SV4 showed the presence of COO- and O-C=O stretching band between 1323 and 1315cm^{-1}, along with SNV3 showing additional COO- stretching vibration at 1610cm^{-1} and SV3, SV4 at 1625cm^{-1}. The samples SNV1, SNV3, SV1 had a O-H bending vibration at band ~948cm^{-1}. The band at 885cm^{-1} belongs to C-C stretch in plane bending vibrations. The absorption frequency at 779cm^{-1} corresponds to O-C=O and Ca-O vibrations. The band around 665cm^{-1} corresponds to out of the plane O-H bending vibrations. The band around 518cm^{-1} is given to Ca-O stretching vibrations. The triply degenerate band at 1458cm^{-1} is weak in NH^+ (**Fig 1 & 2**) **(Table 2 & 3)**.

The results of X-ray diffraction studies of all the chosen samples are shown below **(Fig 3)**. The obtained diffraction patterns were fitted with standard patterns and the corresponding 20 values are tabulated in the **Table 4**. Imageries captured from urinary stone samples by scanning electron microscopy (SEM) **(Fig 4)** assures the X-ray diffraction results. This technique allows a morphological analysis of the stone exterior surface and provides an information about the nature of crystalline compounds, shape of the crystal, internal structure, and some data about relations between crystals and matrix. The scanning electron microscopy of SNV1 and SV2 represented at different dimensions *i.e.,* 1μm, 2μm, 5μm, 10μm and 500nm.

Discussion

It is familiar that the variation in chemical nature and formation of kidney stones are influenced by age and sex of an individual,[16] and has a higher incidence in men, almost twice as high than in females.[17, 18] Analogous to these earlier reports, in our study thirty patients treated, 24 were male (80%) and 06 were female (20%) with an age ranging between 11 to 50 years. One usual observation is the formation of urinary stone was prevalent in patients between 40-50 years, which could be because of regular urinary tract infection which was also reported by previous studies.[12] Association between kidney stone formation and dietary practices cannot be over looked; specially people consuming more animal proteins in their food.[19] Our study showed 66.7% of patients who are non-vegetarians which is like the earlier study.[20] The of biochemical analysis of all renal calculi sample displayed the evidence of ammonia, oxalate and cystine in all the samples followed by calcium and potassium as a slight occurrence. It was also evident that, presence of carbonate, uric acid and magnesium was least, which was alike earlier studies.[12] Further, for the spectroscopic analysis, eight samples from age group 40-50 years and 21-30 years (encompassing maximum patients) were examined.

The FTIR spectra of three samples showed absorption frequencies at 1610cm^{-1} (SNV3), 1625cm^{-1} (SV3, SV4) corresponding to asymmetric COO- stretching vibration.[21,22] A band between 1323cm^{-1} and 1315cm^{-1} belonging to COO- and O-C=O stretching, observed in samples of SNV1, SNV3, SV1, SV3 and SV4.[23, 24] A band ~948cm^{-1} of calcium oxalate monohydrate (COM) arise due to O-H bending vibration (SNV1, SNV3, SV1).[22] The bands between 3493cm^{-1} and 3056cm^{-1} arising due to symmetric and asymmetric O-H stretching (SV3) appears smooth broad band in calcium oxalate dehydrate (COD) SV4.[22, 23, 24] The frequencies of NH_4^+ molecule is weak near the region 3500-3000cm^{-1}.[11] The samples SNV1, SV1, SV3 have strong band near the region 1650cm^{-1} which is assigned to magnesium ammonium phosphate hexahydrate (MAPH)'s component. The band at 565cm^{-1} in SNV1 and

SV1 is assigned to MAPH. The frequencies of the PO^{-34} ion at 569cm^{-1} arises because of triply degenerate deformation mode (SNV4).[24] The plenteous of uric acid as an only mineral component is unique and is found in samples SNV2 and SV2. The uric acid shows bands in range between 3010cm^{-1} to 2600cm^{-1}: bands appear at 3018cm^{-1}, 2803cm^{-1}, 2607cm^{-1} and 2609cm^{-1}. The characteristics band at 1689cm^{-1} and 1681cm^{-1} correspond to carbonyl of (C=O) urea group and band at 1589cm^{-1} attributed to carbonyl of conjugated amid. The uric acid shows band at 1483cm^{-1} and 1485cm^{-1} due to C=N stretch. The band at 1435cm^{-1} is observed in sample SNV4 is due to C=N-H and C-C stretch. The typical band at 1402cm^{-1}, 1404cm^{-1}, 1307cm^{-1}, 1309cm^{-1} corresponds to C=N-H of uric acid. The bands at 1122cm^{-1}, 991cm^{-1} and 619cm^{-1} correspond to ring vibration of uric acid. [11, 22] All the bands indicated in table is observed in spectra of SNV2, SNV4, and SV2.

The two samples (SNV1 and SV1) consist of mixture of calcium oxalate (CaC_2O_4), hydroxyapatite ($Ca_5(PO_4)3OH$) while the two samples (SNV2 and SV2) consist of uric acid. The contents of SV3 and SV4 was found to be calcium oxalate dihydrate (COD) while other SNV3 and SNV4 samples contains calcium oxalate monohydrate (COM), uric acid and PCHT (pentacalcium hydroxy triphosphate).[11,22] The crystal structure of calcium oxalate monohydrate consists of radiating crystals in which the growth zones can be observed by concentric linear characteristics. Apatite is found along with COM structure and is present in sample SNV1. The crystal structure of uric acid is of rhomboid structure or in the form of hexagonal shape as seen in sample SV2. The scanning electron microscopy coupled with energy-dispersive spectra (EDX) showed **(Fig 5)** the presence of P and Ca, which suggest as COM and that involving C, N and O peaks consists of uric acid under 20kV voltage and for 30 seconds of live time. [8, 11, 12]

Conclusions

It is essential to analyse mineral deposits in the human body to understand pathophysiological aspects and composition in the field of medicine. This work aimed to learn differences in the composition of urolithiasis and to validate the correlation between age, and gender with kidney stone formation. The aid of FT-IR, XRD, and SEM techniques enabled the presence of calcium oxalate monohydrate, magnesium ammonium phosphate hexahydrate, ammonia, oxalate, cystine, calcium, potassium, calcium oxalate, hydroxyapatite, pentacalcium hydroxy triphosphate, carbonate, uric acid, and chemical functional groups. SEM analysis results confirmed the XRD analyses. The occurrence of urolithiasis found three times more in men than women. The maximum prevalence of urinary stone was observed in the 40-50 age group.

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Figure Legends

Fig 1: FTIR of non-vegetarian patients.

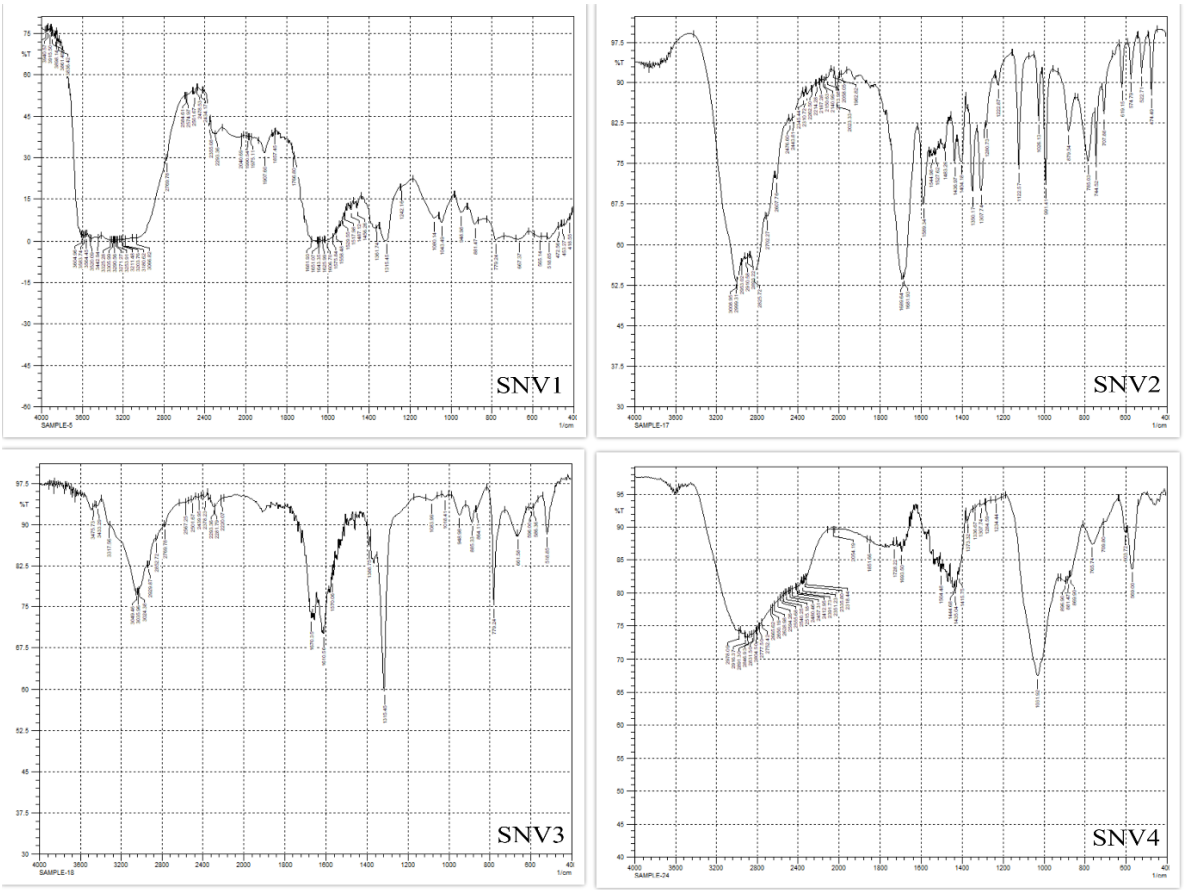


Fig 2: FTIR of vegetarian patients.

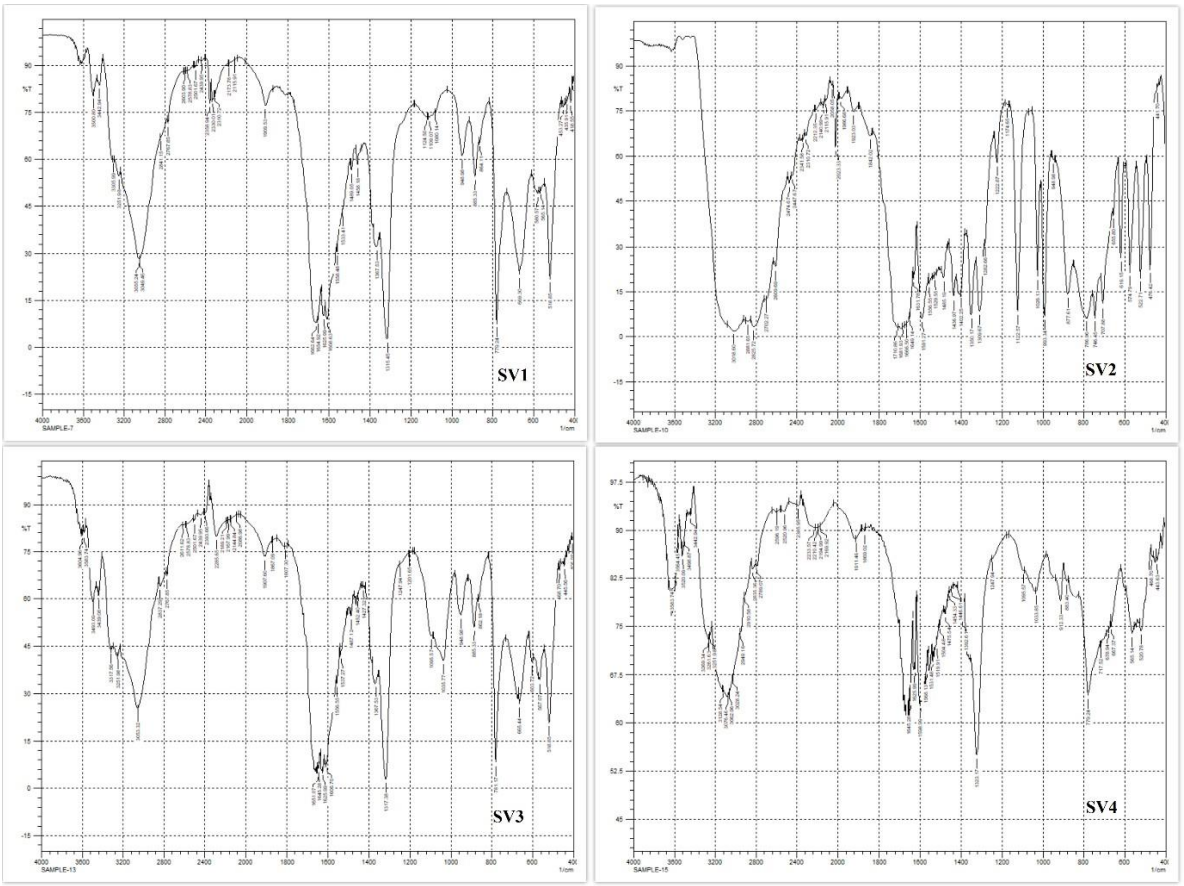


Fig 3: XRD of renal calculi.

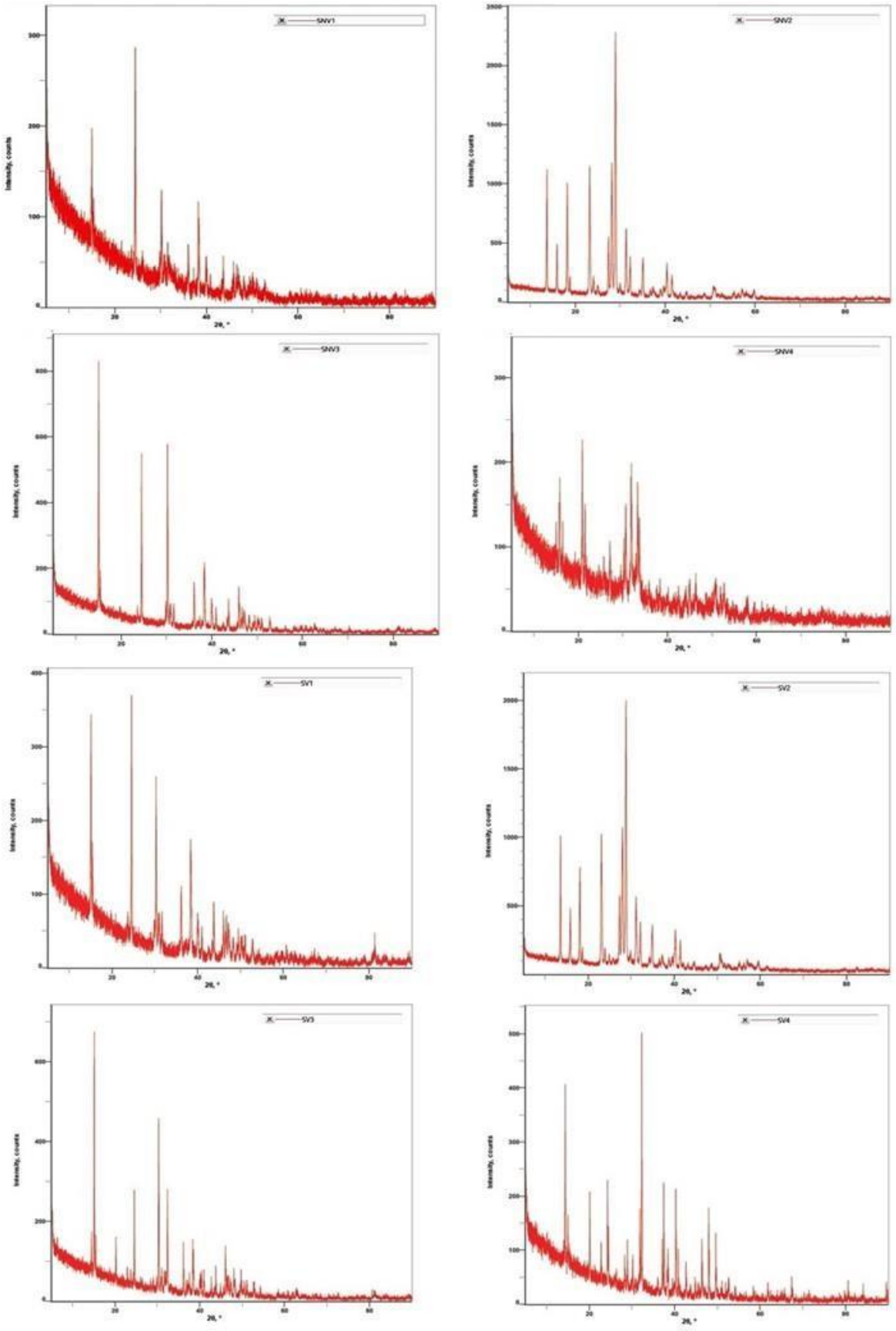


Fig 4: SEM of renal calculi.

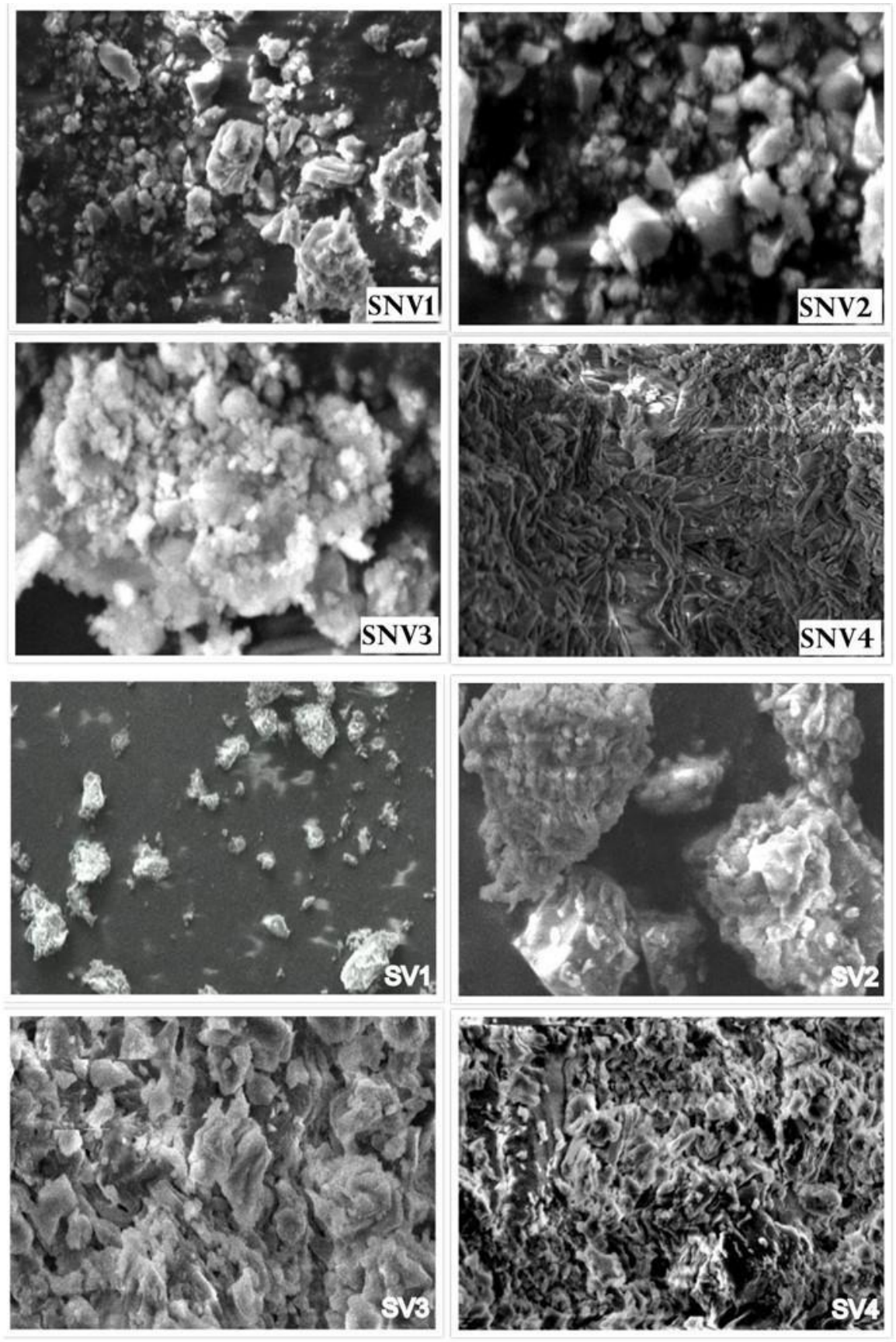
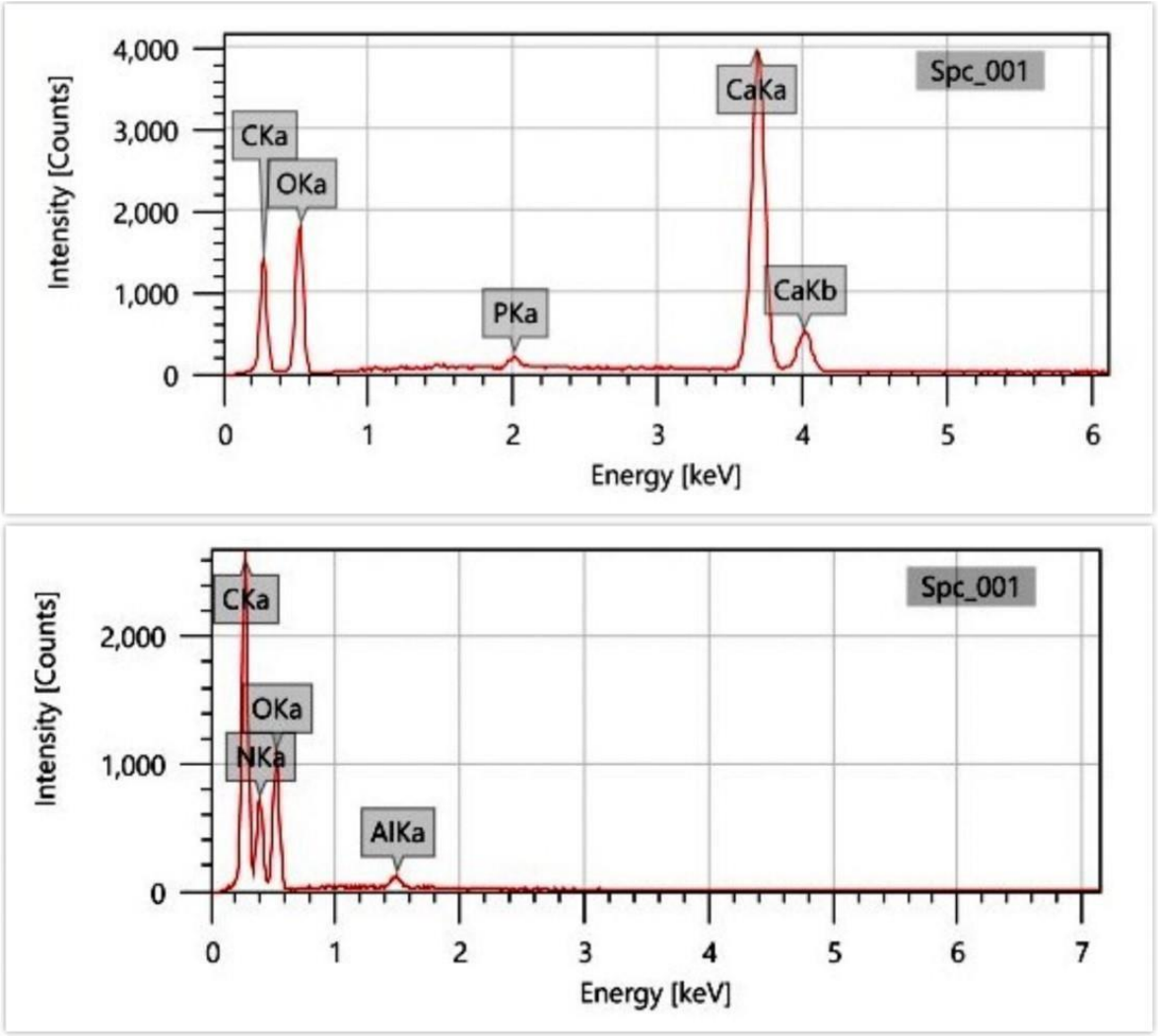


Fig 5: EDX of renal calculi.



Tables

Table 1: Biochemical composition of renal calculi samples.

Samples	Test For						
	Ammonia	Carbonate	Oxalate	Calcium & Potassium	Uric acid	Cystine	Magnesium
1	++	-	++	- / ++	++	++	-
2	++	-	++	-	++	++	-
3	++	+	++	++	++	++	+
4	++	-	++	++ / -	++	++	+
5	++	+	++	++	+	++	+
6	++	-	++	++	++	++	-
7	++	-	++	++	+	++	-
8	++	+	++	++	-	++	-
9	++	-	++	-	-	++	-
10	++	-	++	++	++	++	+
11	++	-	++	-	-	++	-
12	++	-	++	- / ++	-	++	+
13	++	-	++	-	-	++	+
14	++	-	++	-	-	++	-
15	++	-	++	++	-	++	+
16	++	-	++	+ +/ -	-	++	+
17	++	-	++	-	++	++	-
18	++	-	++	++	-	++	+
19	++	-	++	-	-	++	-
20	++	-	++	-	-	++	-
21	++	-	++	-	-	+	-
22	++	+	++	-	-	+	-
23	++	+	++	+ +/ -	-	+	+
24	++	-	++	-	-	+	+
25	++	-	++	++/-	-	+	-
26	++	-	++	- / ++	-	++	+
27	++	-	++	-	-	++	+
28	++	-	++	+ +/ -	++	++	+
29	++	-	++	-	-	++	+
30	++	-	++	+/-	-	++	-

Table 2: FT-IR spectral data of urinary stones.

Sample	SNV1	SNV2	SNV3	SNV4	SV1	SV2	SV3	SV4	Assignment
1.	3600 - 3060	-	-	-	3500 - 3000	-	-	-	(NH_4^+):MAPH
2.	-	-	-	-	-	-	3493 and 3056	-	(O-H):COM
3.	-	3010 - 2600	-	-	-	3010 - 2600	-	-	(C=N-H):UA
4.	-	1689	-	-		1681	-	-	(C=O):UA
5.	1651	-	-	-	1654	-	1651	-	(NH_4^+):MAPH
6.	-	-	1610	-	-	-	1625	1625	(COO^-):COM
7.	-	1589	-	-		-	-	-	(C=N) + (C=C):UA
8.	-	1483	-	-	-	1485	-	-	(C=N):UA
9.	-	-	-	-	1458	-	-	-	(NH_4^+):MAPH
10.	-	-	-	1435	-	-	-	-	(C=N-H) + (C-C):UA
11.	-	1404	-	-	-	1402	-	-	(C=N-H):UA
12.	1315	-	1315	-	1315	-	1317	1323	(COO-) + (O-C=O):COM
13.	-	1307	-	1307	-	1309	-	-	(C=N-H):UA
14.	-	-	-	1234	-	-	-	-	(C-O) +(O-H):UA
15.	-	1122	-	-	-	1122	-	-	Ring vibration:UA
16.	1080	-	-		-	-	-	-	(PO_4^{-3}):MAPH
17.		1025	-	1031	-	1026	-	-	(C-O) +(O-H):UA
18.	-	-	-	-	1109	-	-	-	(PO_4^{-3}):MAPH
19.	-	991	-	-	-	993	-	-	Ring vibration:UA
20.	948	-	948	-	948	-	-	-	(O-H):COM

21.	-		870	-		881	-		877	-		-	(NCN):UA
22.	-		-		885	-		-		-	885	883	(C-C):COM
23.		779	-		779			779	-		781	779	(O-C=O) + (Ca-o):COM
24.	-		-		-	-		-		746	-	-	(C-N):UA
25.	-		-		-	-		-		707	-	-	(O=CN) + (NCN): UA
26.		667	-		661			-	-		665	667	(C-C):COM
27.			619			603	-		619	-		-	Ring vibration:UA
28.	-		-		-	569	-		-		-	-	(PO_4^{-3}): PCHT
29.		565	-		-			565	-		-	-	(OPO): MAPH
30.		518			518	-		518	-		518	520	(Ca-O):COM

Table 3: Identified mineral components of IR spectra in renal calculi (SNV1-SNV4) and (SV1-SV4).

Sl. No.	Samples	Identified minerals
1.	SNV1	COM, MAPH
2.	SNV2	UA
3.	SNV3	COM
4.	SNV4	UA, PCHT
5.	SV1	COM, MAPH
6.	SV2	UA
7.	SV3	COM, MAPH
8.	SV4	COM

Table 4: XRD spectral data of urinary stones.

Sl. No.	SNV1	SNV2	SNV3	SNV4	SV1	SV2	SV3	SV4
1	-	13.68	-	-	-	13.58	-	-
2	14.99	-	-	14.99	-	-	14.48	14.37
3	-	15.94	15.02	-	15.09	15.86	15.1	14.97
4	-	18.18	-	-	-	18.09	-	-
5	-	-	19.80	19.22	19.82	-	-	-
6	-	-	-	20.86	-	-	20.2	20.11
7	-	23.19	-	-	-	23.94	-	-
8	-	-	-	-	24.54	-	24.55	24.26
9	-	-	-	-	-	-	-	24.45
10	-	27.37	-	-	-	27.29	-	-
11	-	28.13	-	-	-	27.99	-	-
12	-	28.9	-	-	-	28.77	-	-
13	-	-	-	29.52	-	29.9	-	-
14	30.17	-	30.92	30.18	30.98	-	30.27	30.2
15	31.53	31.3	-	30.62	-	31.13	-	-
16	-	32.17	-	-	-	-	-	32.24
17	-	-	-	-	-	34.9	-	-
18	-	35.05	-	-	-	-	-	-
19	36.01	-	36.07	-	36.13	-	36.14	-
20	38.12	-	38.34	38.27	38.33	-	38.3	38.4
21	40.82	40.33	-	-	40.08	-	-	-
22	-	41.48	-	-	-	-	-	-
23	-	42.42	42.25	-	42.01	4218	-	42.33
24	43.11	-	43.02	43.05	43.22	-	43.17	-
25	44.15	44.51	-	-	-	44.00	-	44.17
26	-	45.69	-	45.12	-	-	-	45.01
27	-	-	46.02	46.87	46.11	-	46.63	-
28	47.14	-	47.32	47.19	-	47.77	47.60	-
29	48.23	48.45	-	48.19	-	48.48	-	48.55
30	-	49.66	49.20	-	49.87	49.14	49.22	-