

Clinicochemical Profile and Fourier Transform Infrared Spectroscopy Analysis of Urinary Calculi: A Cross-sectional Study

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ABSTRACT

Introduction: Urolithiasis affects approximately 10% of the population in industrialised nations and is associated with significant morbidity and economic burden. The composition of urinary stones varies geographically and influences management strategies.

Aim: To evaluate the clinical profile and stone composition of urolithiasis in patients using Fourier Transform Infrared (FTIR) spectroscopy, the study determined the chemical constituents of urinary stones and assessed the demographic pattern, history of stone recurrence, co-morbidities, and metabolic abnormalities in this urban Western Indian cohort.

Materials and Methods: This cross-sectional study was conducted at the Department of Urology, Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai, Maharashtra, India from January 2019 to August 2021. A total of 300 patients who required surgical intervention for urinary stones {cystolithotripsy, ureteroscopy, or Percutaneous Nephrolithotomy (PCNL)} were enrolled. Demographic data, clinical presentation, co-morbidities, blood biochemistry, urine analysis, and urine culture results were recorded. The stone composition was analysed using FTIR spectroscopy. Data were analysed using descriptive statistical methods.

Results: The study included 187 males (62.33%) and 113 females (37.67%), with patients most commonly aged 31-40

(n=86, 28.67%) or 41-50 years (n=76, 25.33%); 241 (80.33%) had a history of stone disease, and common co-morbidities were hypertension (n=47, 15.67%) and diabetes mellitus (n=34, 11.33%). Serum creatinine was elevated (1.71 ± 1.04 mg/dL), while serum calcium (8.646 ± 0.894 mg/dL) and phosphorus (2.864 ± 0.876 mg/dL) were normal; pyuria (n=180, 60%), haematuria (n=162, 54%), and positive urine cultures (n=58/177, 32.77%) with *Enterococcus* (n=14, 4.67%) and *Pseudomonas* (n=12, 4.00%) were documented. Twenty-four-hour urinary calcium (164 ± 40 mg/24h), phosphorus (459 ± 300 mg/24h), and uric acid (526 ± 120 mg/24h) were within standard reference ranges. FTIR analysis revealed all stones were mixed; major components- quantified independently per stone-included calcium oxalate monohydrate ($57.76 \pm 20.99\%$), calcium phosphate ($44.40 \pm 18.95\%$), calcium oxalate dihydrate ($42.92 \pm 18.89\%$), and ammonium urate ($25.95 \pm 16.14\%$), with totals exceeding 100% reflecting overlapping quantification in mixed stones.

Conclusion: Calcium oxalate monohydrate is the predominant constituent of urinary stones in the Mumbai population, with a peak incidence in the fourth decade of life and a male predominance. The high proportion of patients with a history of stone disease (80.33%) and associated metabolic abnormalities underscore the importance of stone analysis in guiding preventive strategies to reduce the disease burden and recurrence.

Keywords: Calcium oxalate, Kidney calculi, Spectrum analysis, Urolithiasis

INTRODUCTION

Urolithiasis is a common urological condition affecting approximately 10% of the population in industrialised nations, with a rising incidence globally [1-3]. The disease has been recognised since ancient times, with Sushruta first describing stone removal via the urethra in the sixth century BC [4]. Despite advances in surgical management, stone disease remains a significant health burden because of its high recurrence rate, which approaches 50% within 5-10 years of the initial episode [5].

The pathogenesis of urinary stones involves urinary supersaturation with lithogenic substances, resulting from a complex interplay of dietary, environmental, genetic, and metabolic factors [6]. Decreased fluid intake, which leads to concentrated urine, remains one of the most important modifiable risk factors [7]. Additional risk factors include obesity, hypertension, diabetes mellitus, and metabolic syndrome, all of which have shown an increasing prevalence globally [8,9].

Geographical variations in stone composition and incidence have been documented [10]. In India, the prevalence of urolithiasis varies significantly across regions, with particularly high rates reported in

Rajasthan and other parts of Western India, which fall within the country's recognised stone belt [11,12]. These variations reflect differences in climate, dietary habits, water quality, and genetic predispositions [13].

Stone composition analysis is fundamental in understanding the underlying pathophysiology and guiding preventive management [14]. While calcium oxalate stones predominate worldwide, constituting 70-80% of all stones, the relative proportions of monohydrate and dihydrate forms, as well as the presence of phosphate, urate, and other components, vary considerably [15]. Knowledge of the stone composition enables targeted dietary and pharmacological interventions to reduce the risk of recurrence [16].

Mumbai, the capital city of Maharashtra and India's financial hub, has a diverse population with varying dietary habits and lifestyle. Comprehensive data on stone composition and clinical profiles of patients with urolithiasis from this region are limited, as evidenced by a literature review confirming the paucity of FTIR-based studies specifically from this urban centre [11,16,17]. Thus, this study aimed to evaluate the clinical profile and analyse the chemical composition of urinary stones in 300 patients from Mumbai, India, to provide

region-specific data that can guide preventive strategies and reduce the disease burden.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Urology, Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai, Maharashtra, India, over a 32-month period from January 2019 to August 2021. The study was approved by the Institutional Ethics Committee (IEC/080/0021), and written informed consent was obtained from all participants prior to their enrolment.

Sample size: All patients presenting to the urology outpatient department with urinary tract stones requiring surgical intervention were screened for eligibility. Consecutive sampling was performed until the target sample size of 300 patients was achieved. The target sample size of 300 patients was determined based on the feasibility of enrolling consecutive patients undergoing surgical stone removal over the defined 32-month study period at a single high-volume tertiary care centre. This sample size was comparable to or larger than similar prospective FTIR-based stone composition studies from India, which have ranged from 50 to 200 patients [11,16,17]. No formal a priori power calculation was performed as the study was descriptive in nature.

Inclusion criteria: Patients of all age groups and both sex, patients with kidney, ureteric, or bladder stones requiring surgical intervention {cystolithotripsy, Ureteroscopic Lithotripsy (URSL), or PCNL} were included in the study.

Exclusion criteria: Patients managed conservatively without surgical intervention, patients treated with Extracorporeal Shock Wave Lithotripsy (ESWL) alone and patients who declined to participate in the study were excluded from the study.

Clinical evaluation: All enrolled patients underwent comprehensive preoperative evaluation, including a detailed medical history with emphasis on past history of stone disease followed by a complete physical examination. Laboratory investigations comprised urine analysis (routine and microscopy), urine culture and serum biochemistry covering urea, creatinine, calcium, phosphorus, uric acid, sodium, potassium, and magnesium. In addition, 24 hour urinary biochemistry was performed to assess calcium, phosphorus, and uric acid excretion.

Imaging studies: All patients underwent X-ray Kidney-Ureter-Bladder (KUB) and ultrasonography KUB. Contrast-enhanced Computed Tomography (CECT) urography was performed in patients with serum creatinine ≤ 1.4 mg/dL. For patients with serum creatinine > 1.4 mg/dL, non CECT KUB was performed followed by renal scintigraphy (DTPA scan or EC scan) to assess functional renal status. Hounsfield units were recorded for all stones that were visualised on CT. Patients with isolated bladder stones did not undergo CT imaging.

Surgical management: Treatment decisions were based on stone characteristics (size, location, and number) and clinical presentation. Patients with positive urine cultures received appropriate antibiotic therapy prior to the intervention. All patients received prophylactic antibiotics preoperatively. Surgical procedures were performed under general or regional anaesthesia using:

- Cystolithotripsy for bladder stones;
- URSL for ureteric stones;
- PCNL for renal stones.

Intracorporeal lithotripsy was performed using either a Holmium: YAG laser lithotripter or a pneumatic lithotripter, depending on the stone location and availability.

Stone Analysis

Stone collection protocol: Extracted stone fragments were collected intraoperatively across all surgical procedures (PCNL, URSL, cystolithotripsy). While PCNL typically yielded larger fragments (> 2 mm), URSL and cystolithotripsy often produced

smaller fragments. To ensure reliable FTIR analysis, only fragments ≥ 1 mm in any dimension were submitted for spectroscopic examination; smaller dust-like particles were excluded due to potential for inadequate spectral quality. All collected fragments were washed with distilled water to remove blood and debris, and air-dried at room temperature.

FTIR spectroscopy protocol: Stone analysis was performed analysed using FTIR spectroscopy at the central laboratory. Air-dried stones were ground into a fine powder using an agate mortar and pestle. Approximately, 1-2 mg of stone powder was mixed with 200-300 mg of potassium bromide (KBr) and pressed into a transparent pellet under hydraulic pressure. Infrared spectra were recorded in the range of $4000-400$ cm^{-1} at a resolution of 4 cm^{-1} with 16-32 scans per sample. Spectra were compared with a reference library of known stone components for identification. Semiquantitative estimation of individual components was performed using peak area integration relative to the total area of all identified peaks. All analyses were performed by a single experienced technician, and a subset of 10% of samples was independently validated by a second analyst to ensure inter-observer reliability, consistent with recommended protocols for comprehensive morpho-constitutional stone analysis [18].

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all the variables. Continuous variables were expressed as mean $\pm$ Standard Deviation (SD), and categorical variables were expressed as frequencies and percentages. As the study was descriptive in nature, no inferential or comparative statistical testing was performed.

RESULTS

A total of 300 patients with urinary stones requiring surgical intervention were enrolled in the study. The mean age of the study population was 42.3 ± 14.8 years (range: 8 months to 82 years). The most commonly affected age group was 31-40 years ($n=86$, 28.67%), followed by 41-50 years ($n=76$, 25.33%). Together, patients in the 31-60 years age bracket constituted 68.67% ($n=206$) of the study population. Male predominance was observed with a male-to-female ratio of 1.65:1 (187 males, 62.33%; 113 females, 37.67%). A significant majority of patients ($n=241$, 80.33%) reported a history of previous stone disease, representing the proportion of patients with recurrent stone formation at the time of presentation rather than a prospectively observed recurrence rate.

Hypertension ($n=47$, 15.67%) and diabetes mellitus ($n=34$, 11.33%) were the most common co-morbidities, with 6% ($n=18$) of patients having both. The majority of patients ($n=195$, 65%) had no identifiable co-morbidities [Table/Fig-1].

Co-morbidity	n (%)
No co-morbidity	195 (65.00)
Hypertension (HTN)	47 (15.67)
Diabetes mellitus (DM)	34 (11.33)
DM + HTN	18 (6.00)
Chronic Kidney Disease (CKD)	1 (0.33)
CKD + HTN	1 (0.33)
Aplastic anaemia	1 (0.33)
Bilateral Emphysematous Pyelonephritis + DM + HTN	1 (0.33)
Carcinoma Colon	1 (0.33)
Intermittent Haemodialysis (IHD)	1 (0.33)

[Table/Fig-1]: Distribution of co-morbidities ($n=300$).

The mean serum creatinine was mildly elevated (1.71 ± 1.04 mg/dL), while mean serum calcium (8.646 ± 0.894 mg/dL), serum phosphorus

(2.864 ± 0.876 mg/dL), and serum magnesium (1.662 ± 0.212 mg/dL) were within normal limits [Table/Fig-2]. Microscopic pyuria was observed in 180 (60%) patients and microscopic haematuria in 162 (54%) patients.

Parameter (Normal range)	Minimum	Maximum	M $\pm$ SD
Blood urea (10-45 mg/dL)	12	96	25.91 $\pm$ 14.51
Serum creatinine (0.6-1.3 mg/dL)	0.6	5.4	1.71 $\pm$ 1.04
Serum calcium (8.5-10.5 mg/dL)	4.1	11	8.646 $\pm$ 0.894
Serum phosphorus (2.5-4.5 mg/dL)	1.9	8.1	2.864 $\pm$ 0.876
Serum uric acid (2.4-7.2 mg/dL)	2.2	14.8	5.81 $\pm$ 1.209
Serum sodium (130-145 mEq/L)	125	149	135.8 $\pm$ 4.869
Serum potassium (3.5-5.5 mEq/L)	3.0	5.4	4.344 $\pm$ 0.500
Serum magnesium (1.7-2.2 mg/dL)	1.2	2.1	1.662 $\pm$ 0.212

[Table/Fig-2]: Blood biochemistry profile.

Among the patients who underwent urine culture (n=177), significant growth was present in 58 patients (32.77%), of which 55 were bacterial and 3 were fungal (*Trichosporon* species). *Enterococcus* species (n=14, 4.67%) and *Pseudomonas* species (n=12, 4.00%) were the most common isolates [Table/Fig-3].

Organism isolated	n (%)
No growth	119 (39.67)
Not done	123 (41.00)
<i>Enterococcus</i> species	14 (4.67)
<i>Pseudomonas</i> species	12 (4.00)
<i>Escherichia coli</i>	10 (3.33)
<i>Acinetobacter</i> species	8 (2.67)
<i>Klebsiella pneumoniae</i>	8 (2.67)
Gram negative bacilli	3 (1.00)
<i>Trichosporon</i> species	3 (1.00)

[Table/Fig-3]: Urine culture results (n=300).

The mean 24-hour urinary excretion of calcium (164 ± 40 mg/24h), phosphorus (459 ± 300 mg/24h), and uric acid (526 ± 120 mg/24h) were within standard laboratory reference ranges [Table/Fig-4].

Parameter	Number of patients	Minimum	Maximum	M $\pm$ SD
Urinary calcium (100-300 mg/24h)	300	16	185	164 $\pm$ 40
Urinary phosphorus (400-1300 mg/24h)	300	251	2504	459 $\pm$ 300
Urinary uric acid (250-750 mg/24h)	300	450	660	526 $\pm$ 120

[Table/Fig-4]: Twenty-four-hour urinary biochemistry.

Calcium oxalate monohydrate was the predominant component (57.76 $\pm$ 20.99%), followed by calcium phosphate (44.40 $\pm$ 18.95%) and calcium oxalate dihydrate (42.92 $\pm$ 18.89%). All stones analysed were of mixed composition, with no pure stones identified in this study [Table/Fig-5]. The distributions of the major stone components are illustrated in [Table/Fig-6].

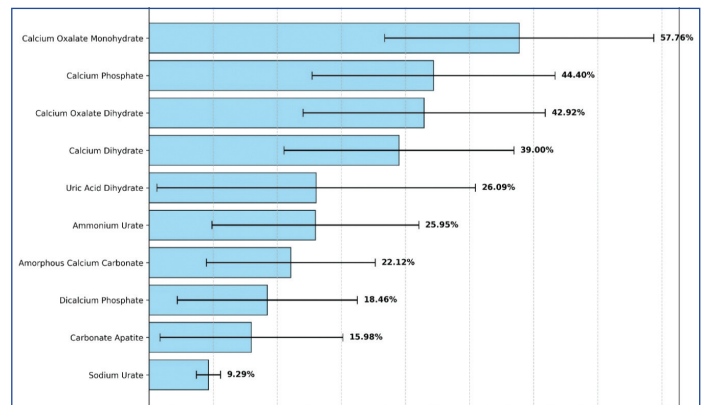
DISCUSSION

This cross-sectional study of 300 surgically managed patients with stones from Mumbai provided comprehensive data on the demographic, biochemical, and FTIR-based compositional profiles of urolithiasis in an urban Western Indian population. The findings reinforce established epidemiological patterns while highlighting region-specific nuances with direct implications for preventive healthcare.

Component	Minimum (%)	Maximum (%)	M $\pm$ SD (%)
Calcium oxalate monohydrate	5	100	57.76 $\pm$ 20.99
Calcium oxalate dihydrate	3	97	42.92 $\pm$ 18.89
Calcium phosphate	10	60	44.40 $\pm$ 18.95
Ammonium urate	3	78	25.95 $\pm$ 16.14
Uric acid dihydrate	10	85	26.09 $\pm$ 24.85
Carbonate apatite	2	60	15.98 $\pm$ 14.27
Amorphous calcium carbonate	10	80	22.12 $\pm$ 13.17
Sodium urate	5	10	9.29 $\pm$ 1.89
Dicalcium phosphate	10	50	18.46 $\pm$ 14.05
Calcium carbonate [†]	40	40	40.00 $\pm$ 0

[Table/Fig-5]: Stone composition analysis by FTIR spectroscopy.

[†]Calcium carbonate was detected in only a single stone, where it constituted 40% of the total composition; therefore, the mean is 40% with a SD of zero and cannot be generalised to the overall cohort; The sum of mean percentages exceeds 100% because all stones were of mixed composition and multiple components were identified in each stone; The percentages for each component represent the mean proportion of that component across all stones in which it was present, not relative percentages that sum to 100%; This is consistent with the methodology where each component is quantified independently relative to the total stone matrix



[Table/Fig-6]: Bar diagram showing mean percentage composition of major stone components.

The peak incidence in the fourth decade (31-40 years), with 68.67% of patients between 31 and 60 years, was consistent with earlier Indian series. Studies from Karad, Maharashtra [11], and Punjab [16] reported the highest prevalence in the 30-60-year age group. Internationally, the 40-49-year peak documented across Korea, the United States, and Japan [2] persists in contemporary analyses [19]. The concentration of the disease during economically productive years underscores its substantial socio-economic burden. The relatively smaller proportions of paediatric and geriatric patients likely reflect divergent aetiologies: paediatric urolithiasis is often driven by metabolic abnormalities, urinary tract infections, and anatomical anomalies [17], whereas geriatric stones are commonly associated with immobility, multimorbidity, and polypharmacy [20].

The male-to-female ratio of 1.65:1 aligns with global trends but is less skewed than the ratios of 3.3:1 reported from Punjab [16], 2:1 from Aurangabad [21], and 2.3:1 from Karad [11]. International comparisons range from 2.5:1 in Japan [22] to 1.15:1 in Iran [23]. The narrowing gender gap has been attributed to the rising rates of obesity and metabolic syndrome in women, both of which are established risk factors for stone formation [8]. Mechanistically, androgen-driven enhancement of hepatic oxalate production and oestrogen-mediated inhibition of calcium oxalate crystallisation are considered key contributors to the baseline male predominance [24,25].

A history of previous stone episodes was documented in 80.33% of patients, substantially exceeding the generally cited 50%, 5-10-year recurrence risk [5]. This likely reflects the referral bias inherent in tertiary surgical units. Hypertension (15.67%) and diabetes

mellitus (11.33%), with 6% exhibiting both, were the predominant co-morbidities, consistent with the well-established bidirectional relationship between nephrolithiasis and cardiometabolic disorders [26]. Madore F et al., demonstrated that a history of kidney stones is associated with an increased risk of hypertension, and Rule AD et al., reported a higher risk of myocardial infarction in stone formers [27,28]. Shared pathophysiological pathways, including insulin resistance, oxidative stress, and endothelial dysfunction, have been proposed to underlie this clustering [29].

Substantial metabolic abnormalities were observed, although causal inference was constrained by the absence of a control group. Mildly elevated serum creatinine levels suggest impaired renal function in a subset of patients, potentially related to obstructive uropathy or recurrent nephrolithiasis, mirroring observations in patients with renal calcifications [30]. Hypercalciuria, the most prevalent metabolic abnormality in patients with calcium stones, is present in 30-60% of cases [31]. The 24-hour urinary data showed mean calcium, phosphorus, and uric acid excretions within standard reference ranges, although these values were lower than the elevated levels reported in stone formers by Hivre MD et al., from Aurangabad [21]. Dietary factors are well-recognised modulators of lithogenic solute excretion [32]; however, their interpretation is limited to literature-derived reference ranges in the absence of a matched control group. Pyuria (60%) and haematuria (54%) reflect the irritative and obstructive properties of calculi, while a 32.77% culture positivity rate, predominantly with *Enterococcus* and *Pseudomonas* species, illustrates the bidirectional nature of infection and stone disease, where stones serve as a nidus for biofilm formation, and urease-producing organisms promote struvite deposition [33].

Calcium oxalate monohydrate was the predominant constituent (57.76±20.99%), consistent with the global predominance of calcium oxalate [15]. Monohydrate stones are typically harder, more resistant to lithotripsy, and associated with hyperoxaluria [34]. The coexisting presence of calcium oxalate dihydrate and a notably high proportion of calcium phosphate (44.40%) exceeds the commonly cited 10-20% range [15] and raises suspicion for distal renal tubular acidosis or primary hyperparathyroidism [35], although dietary and environmental contributions cannot be excluded. The presence of ammonium urate in 25.95% of stones is particularly noteworthy; while rare in industrialised settings, ammonium urate stones are more common in developing regions and are linked to malnutrition, chronic diarrhea, and urinary tract infections [36]. The divergence between this Mumbai cohort and the Karad study, where magnesium ammonium phosphate predominated at 71.2% [11], may reflect geographic variations in dietary patterns, water composition or local infection profiles. All stones were of mixed type, consistent with other Indian reports: Shah A et al., found 94.8% mixed stones [37], Durgawale P et al., reported exclusively mixed heterogeneous calculi [11], and Kaur H et al., observed 90.4% mixed stones, underscoring the multifactorial and dynamic nature of the disease [16].

The high proportion of patients with a history of previous stones necessitates a comprehensive metabolic evaluation following stone clearance. The predominance of calcium oxalate supports interventions such as increased fluid intake, dietary calcium normalisation, sodium and animal protein restriction, and selective potassium citrate use, as recommended by the current clinical guidelines [38]. Stone analysis by FTIR spectroscopy provides detailed qualitative and semiquantitative compositional data essential for individualised prevention [14,39]. According to clinical guidelines [38], calcium phosphate stones warrant investigation for primary hyperparathyroidism and renal tubular acidosis [35], whereas ammonium urate stones require infection control and correction of underlying nutritional or gastrointestinal disorders [36]. Although these guideline-based strategies align

with the observed compositional patterns, their efficacy in this specific population remains to be evaluated in prospective outcome studies.

Limitation(s)

This study had several limitations. Recruitment at a single tertiary centre may introduce a selection bias toward complex or recurrent cases, limiting generalisability. The absence of a control group restricted biochemical comparisons to literature-derived normal ranges. The 24-hour urine panel omitted oxalate, citrate, and magnesium, and collection completeness was not biochemically verified using urinary creatinine. Dietary and fluid intakes were not systematically recorded. The cross-sectional design precluded the assessment of long-term recurrence or intervention effects. The semiquantitative FTIR method, although validated with a 10% subset re-analysis, has inherent limitations for overlapping spectral bands and the detection of minor components.

Longitudinal studies incorporating complete 24-hour urinary risk profiles and systematic dietary inventories across multiple centres are needed. Randomised trials of composition-tailored preventive regimens will establish locally effective strategies. Integrating genetic, water quality and climate variables may further elucidate the unique stone spectrum observed in this region.

CONCLUSION(S)

This cross-sectional study of 300 patients with urolithiasis from Mumbai, India, demonstrated that calcium oxalate monohydrate as the predominant stone constituent, with a peak incidence in the fourth decade of life and male predominance. The high proportion of patients with a history of previous stone disease (80.33%), the presence of mixed stones in all cases, and the observation that 24-hour urinary excretion of calcium, phosphorus, and uric acid fell within standard reference ranges while a subset of patients exhibited elevated serum creatinine, underscore the multifactorial aetiology of nephrolithiasis in this population. Comprehensive 24-hour urine metabolic evaluation remains essential for individualised risk assessment. These findings emphasise the importance of stone analysis using FTIR spectroscopy in guiding targeted preventive strategies to reduce disease burden and recurrence in patients with urolithiasis.

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