Research Article

Comprehensive Analysis of Renal Stones Using FTIR Spectroscopy in a Referral Laboratory in Nepal

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Keywords

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Abstract

Background: Renal stone disease, a prevalent urological disorder, significantly impacts public health in Nepal. Analyzing the composition of renal stones is crucial for understanding their etiology and guiding treatment and prevention strategies. FTIR spectroscopy is a reliable technique for identifying the chemical composition of renal stones. This study aims to analyze the composition of renal stones using FTIR spectroscopy in a referral laboratory in Nepal.

Methods: A total of 300 renal stones from patients were analyzed. The stones were collected, cleaned, and powdered before being subjected to the Thermo Fisher Scientific FTIR Spectrometer. The spectra obtained were compared to the reference spectra to determine the composition of the stones.

Results: The analysis revealed that calcium oxalate monohydrate in 41% and calcium oxalate dihydrate in 29 % were the commonest types. Other less common compositions included uric acid, struvite, and cystine stones.

Conclusion: FTIR spectroscopy effectively identified the composition of renal stones in the studied population. The predominance of calcium oxalate stone highlights the need for targeted prevention and treatment strategies in Nepal.

Introduction

Renal stone is a common disease that affects 5–13% of the population worldwide [1]. In Nepal, the prevalence of renal stones has been increasing and it has caused higher surgical intervention rates. Loss of renal function and urosepsis are potential complication of untreated renal stones. Endourological procedures such as retrograde intrarenal surgery and percutaneous nehrolithotomy have been used recently in the management of renal stone in Nepal [2,3]. However, using Fourier Transform Infrared (FTIR) spectroscopy for the analysis of renal stone in Nepal is uncommon. Prevention of stone recurrence is important to reduce the morbidity and it also leads to reduced costs as fewer interventions are required.

FTIR spectroscopy has emerged as a powerful tool for the detailed analysis of renal stones [4,5]. This method offers precise identification of the stone's chemical composition, allowing for accurate differentiation between various types of stones, such as calcium oxalate monohydrate, calcium oxalate dihydrate, uric acid, and others. Understanding the precise chemical composition of renal stones is crucial for developing effective prevention and treatment strategies. Furthermore, the goal of metabolic evaluation is to prevent recurrent stone formation in high risk stone producers and to prevent extra renal complications in associated systemic disorders.

There are other methods of stone analysis such as wet chemical analysis which can only identify the presence of individual ions and radicals without differentiating a specific compound in stone mixture. Similarly, thermogravimetry requires large amount of material for optimal resolution. Optical polarizing microscopy uses the principle of interaction of polarized light with crystals of stones and it is cumbersome. X- ray diffraction uses monochromatic X-rays for identifying the constituents of stone based on unique diffraction pattern produced by a crystalline material. FTIR spectroscopy determine exact quantitation of stone constituents at moderate cost and is quick in identifying organic components or non crystalline substances, e.g. purine, proteins or fat and drug metabolites [6]. Mixed stones are common, and FTIR spectroscopy can be used to determine the relative percentage content of the various components with high accuracy even with very small amount of sample material.

Approximately 40% of the global population residing in high-risk zones, especially in Asian countries like Saudi Arabia and India, was reported to be affected by urolithiasis in 2000. This prevalence is projected to increase to 50% by 2050 [7]. The European Association of Urology guidelines on Urolithiasis (2013) emphasize the limitations of traditional chemical analysis and strongly recommend FTIR spectroscopy as the preferred method for urinary stone composition analysis [8]. In south Asian countries, FTIR has been widely adopted since the early 2000s for renal stone analysis, with documented use in countries such as India [9], Pakistan [10], China [11], and Sri Lanka [12].

In Nepal, however, the application of FTIR for renal stone analysis is still in its nascent stage, with the first reports emerging only in 2022 [13]. This limited adoption contrasts with the high and rising prevalence of renal stones in Nepal, yet data on the epidemiology and specific composition of kidney stones remain insufficient [14]. The integration of FTIR, utilizing computerized spectrophotometers and comprehensive reference libraries, allows for precise quantitative analysis of stone composition. Expanding FTIR capabilities to more urolithiasis centers across Nepal would enhance diagnostic accuracy and deepen the understanding of the pathophysiology of kidney stones, a crucial step given the increasing trend of renal stone cases in the region. Any additional data gathered through such approaches will be valuable in addressing this significant public health concern.

Our study aims to fill this gap by presenting the results of renal stone analyses conducted using FTIR spectroscopy in a referral laboratory in Nepal. By providing a comprehensive overview of the types and frequencies of renal stones in this population, we hope to contribute valuable insights that can inform better clinical practices and public health policies.

Method

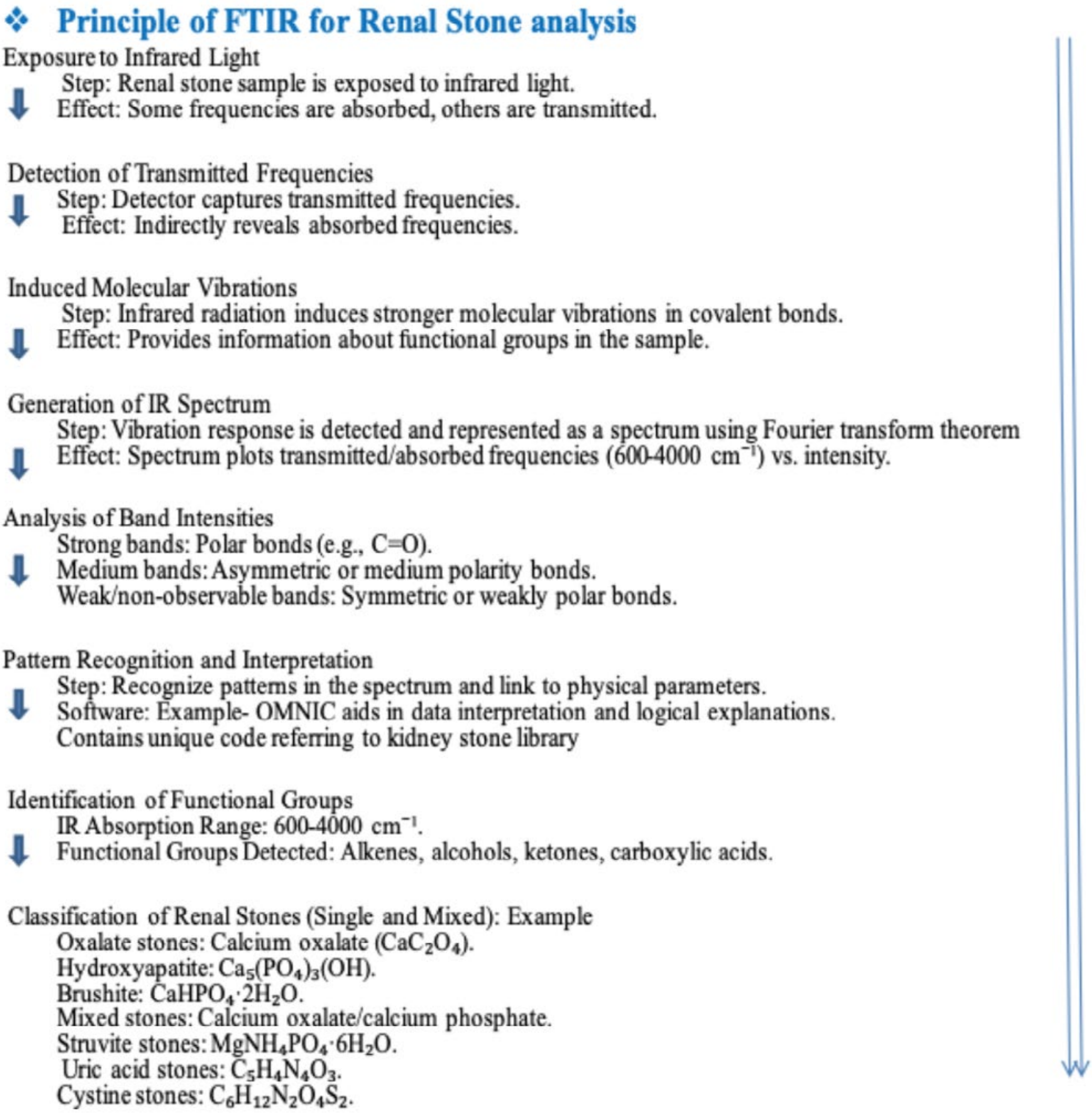
This study was carried out over a period of 28 months starting from January 2021 to May 2023 at Samyak Diagnostic Pvt Ltd thus including all 300 renal stone samples collected in this period. In accordance with institutional and regulatory guidelines, this study utilized secondary laboratory data and does not involve direct interaction with human subjects or the collection of new, identifiable information. This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Stone Analysis

Renal stones were analyzed using Thermo Fisher Scientific FTIR Spectrometer (Model- Nicolet Summit LITE, Serial No: BFJ2010011). All stones were washed with distilled water and dried completely in an incubator. The sample was grounded with a pestle and mortar until a fine homogeneous powder was obtained. For the FTIR spectroscopic investigation, the powdered urinary stone material (20 mg) was homogeneously mixed with potassium bromide (200 mg), an inert carrier, which did not show any absorption in the spectral region to be investigated. The mixture was then transferred to cylindrical hole and the bolt press and kept under the hydraulic press; the pump is used to increase the pressure in the hydraulic press to 15 tons and left for 2-3 minutes. The pressure is released slowly to form the potassium bromide disc or Pellet, which is then, analyzed. The resulting spectrum was then compared with the entire reference spectrum for the known components of stones, allowing a precise analysis of the complex crystal mixture for each crystal type using the OMNIC Paradigm .Ink software (Serial No: 210208422).

In FTIR spectroscopy, photons possessing energy that exactly matches the vibration energy of a covalent bond in stone are absorbed. Thereafter, an infrared spectrum will show which bonds have absorbed radiation (wavelength) and absorption efficiency (intensity). This combination of wavelength and intensity generate a unique fingerprint for each component that can be used for both qualitative and quantitative analysis of renal stone. The details of principle of FTIR for analyzing renal stones is shown in Figure 1.

Figure 1: Details of principle of FTIR for analyzing renal stones.



Statistical Calculation

Descriptive statistics was done in Microsoft excel and results were expressed in frequencies and percentage. Statistical examination of renal stone incidence and composition with respect to sex and age was done using the Chi-square test ($p < 0.05$) in SPSS for Windows version 20.0.

Results

Demographic findings

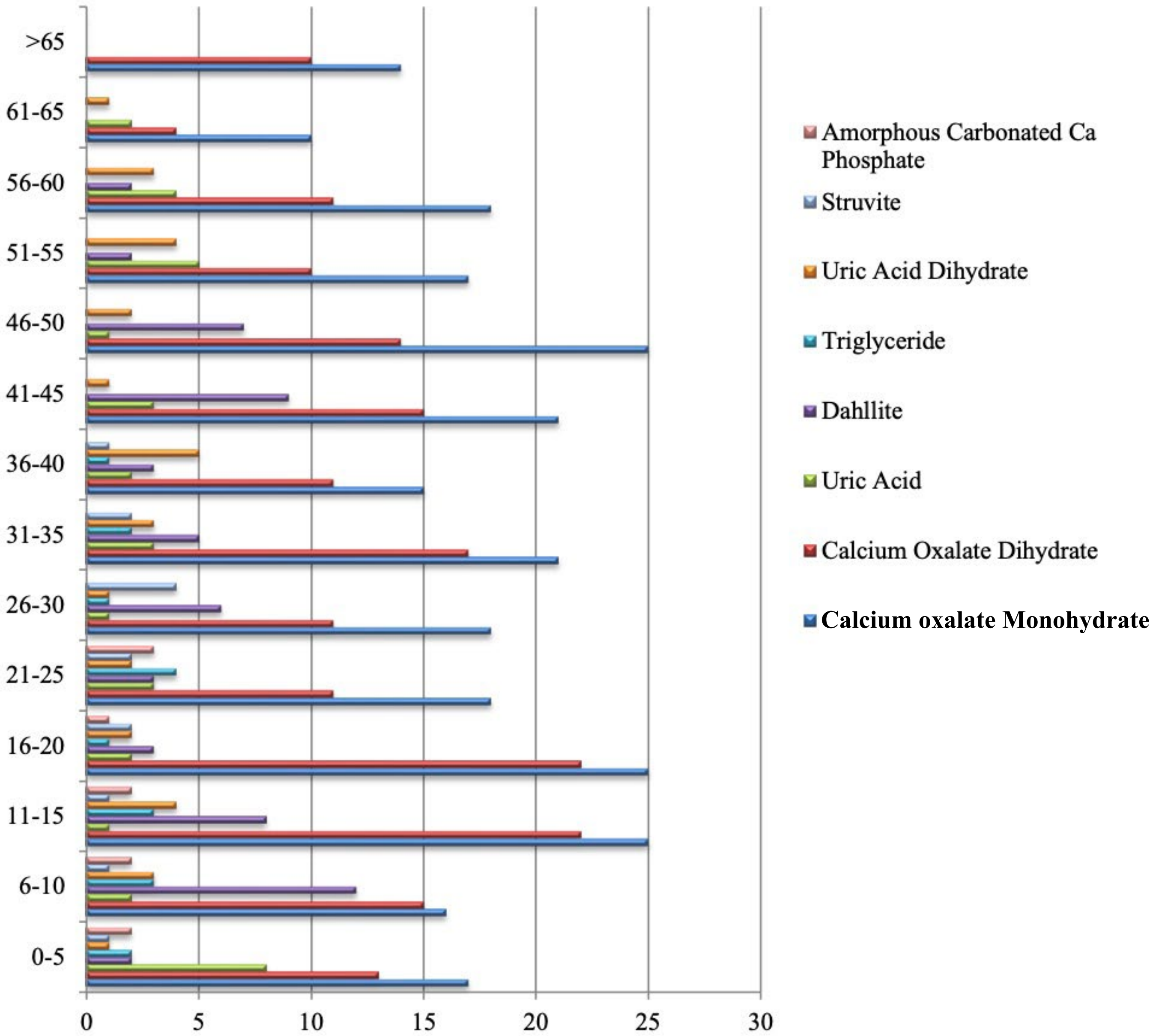
This dataset comprises information of 300 individuals diagnosed with renal stones, categorized by age and gender. The data includes 150 females and 150 males, with ages ranging from 2 to 84 years. The Kolmogorov–Smirnov test yielded a p-value of 0.000, indicating a significant deviation from normality. Therefore, the median age of study participants is 35 years. The youngest male is 2 years, and the oldest male is 84 years old. Age of female ranged from 9 to 77 years. The data indicates that renal stones affect a wide age range, from young children to the elderly. To better understand the age distribution, we categorized the individuals into age groups and analyze the sex distribution within each group as shown in Table 1.

Table 1: Age groups and sample size by sex of renal stones analyzed.

Age Group (Years)	Number of Females	Number of Males	Total
0-10	2	4	6
11-20	4	12	16
21-30	36	36	72
31-40	34	34	68
41-50	26	26	52
51-60	22	24	46
61-70	19	11	30
71-80	5	3	8
81-90	1	1	2
Total			300

The prevalence of renal stones is notably high in the younger population aged 21-30 years, with an equal distribution of 36 males and 36 females. Similarly, in the middle-aged groups (31-40 and 41-50 years), the distribution remains equal between males and females, suggesting that the prevalence of renal stones is consistent across these age groups. A chi-square test was conducted to evaluate the association between sex and age categories, and the results indicated statistically significant difference in sex distribution with higher incidence in males across 11-20 years age group (p-value: 0.002) and higher incidence in female across 61- 70 year age group (P-value: 0.002). We investigated the prevalence of various types of renal stones in relation to age. Our analysis indicates that calcium oxalate stones are the most common across all age groups (Figure 2).

Figure 2: Case distribution (X-axis) of common renal stones among various age groups (Y-axis).



FTIR Spectroscopy Findings

The predominance of calcium oxalate stones, with calcium oxalate monohydrate (COM) comprising 41% and calcium oxalate dihydrate (COD) 29% of cases, aligning with global trends was found in this study. In addition to calcium oxalate stones, our study identified several other types of renal stones, as illustrated in Figure 3. Renal stones with a low incidence, comprising less than 1% of the total, are not depicted in the pie chart. These rare types include ammonium urate, newberyite, 2,8-dihydroxyadenine, brushite, cholesterol, sodium urate monohydrate, calcium phosphate, calcium apatite, and whitlockite. FTIR Spectroscopic characteristic of renal stone components is shown in Table 2.

Figure 3: Distribution of common renal stones in Nepalese population.

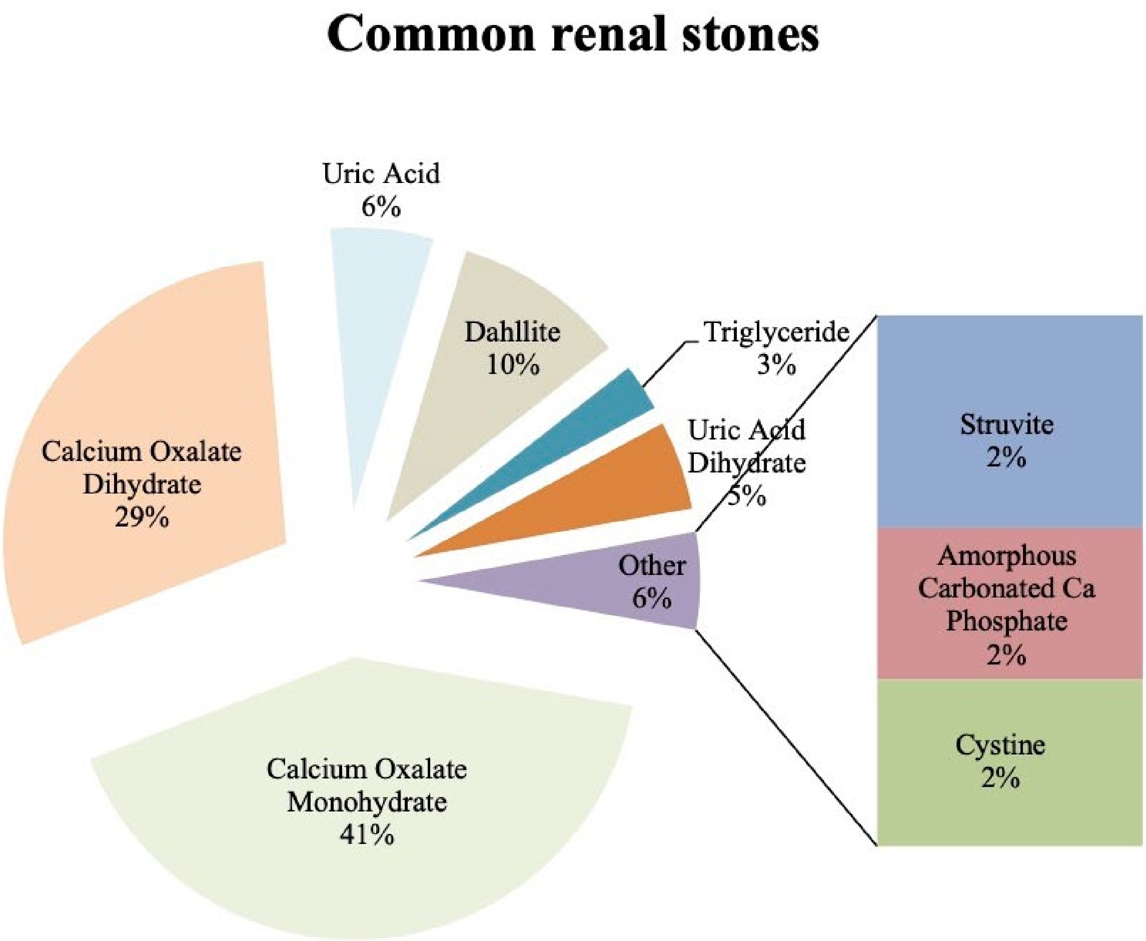
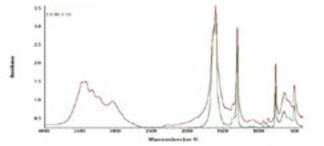
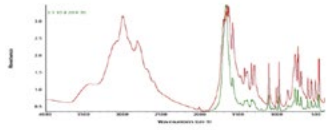
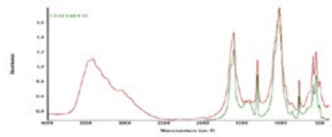
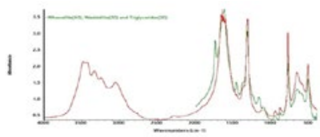
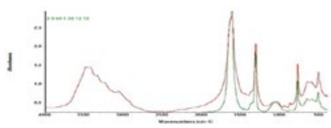
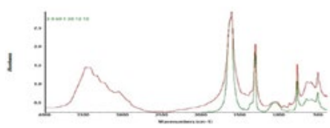


Table 2: FTIR Spectroscopic Characteristics of Common Renal Stone Components in this study.

Compound	Functional Groups	Types of Vibration and Band Assignment	Theoretical Range (cm^{-1})	Reference Spectra
Calcium Oxalate Monohydrate (Whewellite: CaC2O4.H2O)	C=O (carboxyl), O-H (hydroxyl)	ν(C=O) stretching, ν(O-H) bending	1620–1640, 1310–1330	
Calcium Oxalate Dihydrate (Weddellite: CaC2O4.2H2O)	C=O (carboxyl), O-H (hydroxyl)	ν(C=O) stretching, ν(O-H) bending	1320–1370, 780–820	
Uric Acid	C=O (carbonyl), N-H	ν(C=O) stretching, ν(N-H) bending	1600–1650, 1320–1350	
Uric Acid Dihydrate	C=O (carbonyl), N-H	ν(C=O) stretching, ν(N-H) bending	1600–1700, 1300–1400	
Dahllite (Carbonate Apatite)	P-O (phosphate), C-O (carbonate)	ν(P=O) stretching, ν(C-O) stretching	1000–1100, 1410–1460	
Struvite (Magnesium Ammonium Phosphate)	P-O (phosphate), N-H	ν(P=O) stretching, ν(N-H) bending	950–1050, 3200–3400	
Triglyceride	C=O (ester), C-H	ν(C=O) stretching, ν(C-H) bending	1740–1750, 2850–2950	
Amorphous Carbonated Calcium Phosphate	P-O (phosphate), C-O (carbonate)	ν(P=O) stretching, ν(C-O) stretching	1000–1100, 1450–1550	
Cystine	S-H (thiol), N-H, C=O	ν(S-H) stretching, ν(C=O) stretching	2550–2600, 1580–1650	

Discussion

Renal stone analysis of high quality is an essential part of the basic evaluation to identity patients at high risk of recurrent stone disease. In many cases, more extensive examination is required to reveal the etiology of stone formation in the individual patient, for example 24-hour urine measurements of citrate, oxalate, calcium and uric acid.

The present study shows an equal distribution of renal stones among males and females in 21-50 years age group. This finding is in contrast to other studies where male to female ratio is significantly higher [13-15]. Nevertheless, our study shows higher male prevalence in males across 11-20 years.

Identifying the specific types of stones prevalent in a population allows healthcare providers to develop targeted interventions, such as dietary modifications, pharmacological treatments, and public health initiatives aimed at reducing the incidence of stone formation. The major types and incidence of renal stones in this study and the related risk factors and required dietary modifications in each category is shown in Table 3 [16-20].

The higher prevalence of calcium oxalate stones aligns with neighboring countries. The high incidence of calcium oxalate containing renal stones in South Asian countries may be influenced by regional climate, dietary habits, and fluid intake patterns. In Pakistan, the hot climate, combined with factors such as low urine volume, acidic urine pH, and a diet low in protein and calcium but high in oxalate-rich foods, has been shown to promote the formation of uric acid and calcium oxalate stones, as noted by Rizvi et al [15, 21]. Similarly, in India, high rates of calcium oxalate stones are likely driven by a hot climate, oxalate-rich diet, and low fluid intake [9]. Subgroup analysis from an Indian study further demonstrated that the prevalence of calcium oxalate stones increases with age, while uric acid, struvite, and cystine stones decrease [9,15]. This finding is similar to our study. Moreover, hyperoxaluria rates appear significantly higher in Asian countries compared to Western countries (56.8% vs. 23.8%; $p < 0.001$), which highlights how dietary practices and global climate shifts may be key contributors to the rising incidence of renal stones [22]. These factors underscore the importance of targeted prevention strategies that consider regional dietary and environmental influences.

In Nepal, dietary habits rich in oxalate-containing foods such as tea consumption and low fluid intake due to socioeconomic and geographical factors may contribute significantly to this trend [23, 24]. This finding underscores the need for targeted dietary interventions such as minimizing oxalate intake and public health strategies such as advocating for low salt and sufficient water intake to reduce the incidence of these types of stones.

Beyond calcium oxalate stones, our study identified several other types of renal stones. Dahllite stones are indicative of calcium phosphate stones and suggest the importance of managing urinary pH and calcium levels. Similarly, uric acid stones highlight the relevance of metabolic conditions such as hyperuricosuria and low urinary pH. Dietary modifications and medications that alkalinize urine can be effective preventive measures. Likewise, the struvite stones are usually associated with urinary tract infections. The presence of other types of stones, although less frequent, indicates the diversity of metabolic disturbances contributing to urolithiasis in this population. Therefore, these findings emphasize the need for comprehensive metabolic evaluations in patients presenting with renal stones.

Table 3: Major type of renal stones in Nepalese population along with its risk factor and recommendation for its prevention.

Common Renal Stone Types	Distribution Percentage	Risk Factors	Recommendations for Prevention
Calcium Oxalate Monohydrate (COM) and Calcium Oxalate dehydrate (COD)	COM 41% COD 29%	Hypercalciuria (250 mg/day) Hyperoxaluria (> 45 mg/day) Hypocitraturia (< 320 mg/day) Low urine volume (< 2 L/day)	Sufficient water intake~ 2L/day Minimize oxalate intake (spinach, cranberry, grapes, potatoes, nuts,tea,coffee) Consume fruit juice(Citric acid) Sodium restriction (<1500 mg/day)
Dahllite [Calcium Phosphate]	10 %	Hyperphosphaturia (> 1100 mg/day) Alkaline urine pH (> 7.0) along with low urine volume Hypercalciuria and hypocitraturia	Sufficient water intake~ 2L/day Consume Lemon, lime, melon and oranges that contains citric acid

Uric Acid and Uric Acid Dihydrate (UAD)	Uric Acid 6 % UAD 5 %	Low urine volume Hyperuricosuria (> 700 mg/day) Acidic urine pH (< 5.5)	Avoid high-purine foods (organ meats, mushrooms, asparagus, green peas, spinach, fish) Increase plant protein rather than animal protein to increase alkaline load in urine
Struvite (Magnesium ammonium phosphate)	2.0%	Urinary tract infection by a urea - splitting bacterium Neurogenic bladder and anatomic abnormalities of urinary tract	Treatment of UTI Surgery for anatomical defects
Cystine	2.0%	Inherited disorder like Cystinuria	Increase cysteine solubility by increasing fluid intake Sodium restriction Reduce meat intake (contains methionine that is converted to cysteine)

References = [16-20]

This study has certain limitations that should be acknowledged. The sample size, while adequate, may not fully represent the entire population of Nepal. Additionally, as a referral laboratory, our sample may have a selection bias toward more complicated or recurrent cases, and we used convenience sampling technique. Future studies with larger and more diverse populations would be beneficial to validate and expand upon our findings. Additionally, exploring the relationship between dietary habits, socioeconomic status, and stone composition could provide deeper insights into preventive measures. Longitudinal studies to track the recurrence rates and the effectiveness of dietary and medical interventions in patients with different types of stones would also be beneficial.

Conclusion

In conclusion, the analysis of renal stones using FTIR spectroscopy in a referral laboratory in Nepal has revealed that calcium oxalate monohydrate and dihydrate stones are the most prevalent types. This laboratory based data reveals that renal stones are prevalent across all age groups and affect young population equally. These insights can inform targeted prevention and treatment strategies for renal stone disease.

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Conflict of Interest

Authors declare no conflict of interest in the publication of this manuscript.

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