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ESTIMATION OF CALCIUM FROM KIDNEY STONES IN THE PATIENTS OF KHAIRPUR MEDICAL COLLEGE CIVIL HOSPITAL KHAIRPUR, SINDH, PAKISTAN

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Abstract

Our study aimed to estimate the concentration of Calcium (Ca) from the kidney stones of the patients of Khairpur Medical College Civil Hospital Khairpur, Sindh, Pakistan. The research was conducted at the Institute of Chemistry, shah Abdul Latif University Khairpur. The research was done on 100 renal calculi specimens (65 men and 35 women in age range of 15 to 80 years). All the stone samples were examined for composition by using UV-vis double beam spectrometer and FTIR spectrophotometery. In FTIR study we noted that 100 examined calculi specimens comprised 48% of COM, 19% of Uric acid (pure), 4% of Calcium Phosphate, 13% of struvite, 4% cystine and 12% mixed calculi of (CaOx + uric acid and CaOx + struvite stones). COM was the dominating type of calculi with a percentage of 48% followed by Uric acid stones. Analysis of renal calculi based on UV-Vis double beam spectrophotomer revealed that Ca was present in all of the kidney stone specimens. The maximum amount of Ca was found in CaOx stones. The average amount of Ca in CaOx, Mixed, Uric Acid and Struvite stones was 33.4, 8.7, 4.16 and 6.74 mg/g.

Keywords:

Kidney stone, FTIR, Stone composition, Calcium carbonate.

INTRODUCTION

Nephrolithiasis (also known as urolithiasis) is a widespread pathophysiological disorder that occurs when different minerals in the urine become concentrated and crystallize, resulting in the kidney stone formation (Memon, Soomro, Shar, Korai, & Shar, 2020). The chemical composition of kidney stones typically includes Calcium, Magnessium, Ammonium, Uric acid, oxalate, phosphate and other dissolved minerals normally present in urine (Dung, Soomro, Shar, Korai, & Shar, 2020). Elevated concentrations of these substances can lead to crystallization and subsequent stone development. This condition has been observed since antiquity, and despite advances in medical science it remains a significant global health concern, owing to its increasing incidence, high recurrence rates and substantial morbidity (Baatiah et al., 2020; Kachkoul et al., 2023). Estimates suggest lifespan risk of stone creation ranges from 2 % to 20 % across different populations, with Ca-based calculi (especially calcium oxalate and mixed calcium oxalate/phosphate) comprising over 80 % of cases (Kachkoul et al., 2023; Romero, Akpinar, & Assimos, 2010). Epidemiological surveys indicate rising incidence and prevalence trends worldwide, particularly in developed settings and among working age adults (Evan et al., 2005; Ferraro et al., 2013; Yao et al., 2025). Multiple interrelated factors contribute to stone formation, underscoring the multifactorial aetiology of urolithiasis (Ahmad et al., 2025). Key biochemical abnormalities include urinary supersaturation with stone forming solutes such as calcium, oxalate, phosphate and urate, coupled with reduced inhibition by substances like citrate and magnesium (Kravdal, Helgø, & Moe, 2015; Romero et al., 2010). Genetic predisposition also plays a role, with familial clustering and identified gene variants in populations with high incidence (Ahmad et al., 2025; Sobirov, Erniyozov, & Aliqulov, 2025).

Complications may include hydronephrosis, impaired renal function, and in infected cases even sepsis (Kravdal et al., 2015). Size of calculi varies widely, from millimetre sized granules to large stones several centimetres in diameter and the migration of stones or growth within the renal collecting system are recognised as major mechanisms of renal damage (Ahmad et al., 2025).

Diagnosis of urolithiasis relies on a combination of clinical evaluation, laboratory investigation and imaging. Non contrast computed tomography (NCCT) is considered the gold standard for stone detection, while ultrasound and plain radiography (KUB) may be used as alternatives in resource limited settings (Becerra, 2024). Laboratory workup includes urinalysis (hematuria, crystals, infection) and, in recurrent cases, 24 hour urine collections to assess volume, calcium, oxalate, citrate and other parameters (Sakhaee, 2009). Importantly, analyzing the composition of recovered stones provides valuable insights into pathogenesis and guides tailored prevention strategies (Ahmad et al., 2025; Kravdal et al., 2015). Techniques for stone analysis include Fourier Transform Infrared (FT IR) spectroscopy, X ray diffraction, and wet chemical methods; FT IR is increasingly favoured for its accuracy, speed and minimal sample requirement (Aggarwal, Narula, Kakkar, & Tandon, 2013; Kachkoul et al., 2023). Pain relief with non steroidal anti inflammatory drugs (NSAIDs) is first line, alongside hydration and medical expulsive therapy for appropriate cases (Pearle et al., 2014). Minimally invasive procedures, such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS) with laser lithotripsy, and percutaneous nephrolithotomy (PCNL) have revolutionized stone management in many countries (Kachkoul et al., 2023; Kravdal et al., 2015).

Materials and Methods

Patient Population

The study comprised 100 renal calculi patients with 65 males and 35 females (age 15 to 80 years) from Khairpur Medical college civil hospital.

Calculi Analysis

The study analyzed 100 surgically removed renal calculi. The surgically detached kidney stones were air-dried on sterile gauze, rinsed with deionized water and then oven-dried well at 37^0C, then the specimens were weighed. After documenting the stones' physical and morphological characteristics, a single renal calculi specimen of each sufferer was pulverized using a pestle and mortar for five minutes. The finely ground and homogenized powder of calculi specimen was transferred to specimen tubes and maintained in a dark cabinet to protect from light, until analysis. FT-IR Spectrometer (NICOLET iS10) was employed to determine the chemical composition of renal calculi powder. Whereas UV-Visible double beam spectrophotometer 9500 Cecil was used for the determination of percentage/ amount of Calcium..

Preparation of Stock Solutions

A 1000 ppm calcium stock solution was prepared by accurately weighing 0.6243 g of calcium carbonate ($CaCO_3$), which was then dissolved in 3.5 mL of concentrated hydrochloric acid (HCl). The resulting solution was diluted to a final volume of 250 mL using deionized water. Buffer solution with pH 11.4 was prepared by dissolving 1.4 g of 2-amino-2-methyl-1-propanol ($C_4H_{11}NO$) in 100 mL of deionized water. This buffer was used to maintain the optimal pH for complex formation during analysis. The o-CPC reagent was prepared by dissolving 15 g of o-cresolphthalein complexone in 100 mL measuring flagon. This reagent served as the chromogenic agent for colorimetric detection.

Estimation of Calcium from urinary Calculi by UV-Vis Spectrophotometery

Analytical Principle

The determination of calcium ions was based on their ability to form colored complexes with the o-cresolphthalein complexone (CPC) reagent under alkaline conditions. Ca ions produced a deep purple-colored complex upon binding with CPC. To eliminate interferences during analysis, 8-hydroxyquinoline was employed to mask the interference of iron (Ferraro et al.) and magnesium (Mg) during calcium quantification. Spectrophotometric measurements were performed to determine absorbance values corresponding to each complex. The calcium-CPC complex exhibited a maximum absorbance at 570 nm.

FT-IR Spectroscopy Detection

FT-IR Spectrometer (NICOLET iS10) was employed to determine the chemical composition of renal calculi. FT-IR is highly preferred and extensively utilized method for investigating nephrolithiasis. This analytical technique is ideal for characterizing the composition of kidney stone. This method provides rapid and precise results, enabling both qualitative and semi quantitative analysis.

Results and Discussion

The demographic distribution of nephrolithiasis patients in the study is presented according to gender and age. Out of a total of 100 patients, 65 were male and 35 were female, representing 65% and 35% of the total cases, respectively. This notable male predominance is consistent with global epidemiological trends, where men are generally more prone to renal calculi creation because of combination of metabolic, hormonal, and lifestyle factors. When categorized by age, the highest prevalence of nephrolithiasis was observed in the 16–30 age group, which accounted for 38% of the total cases. This was followed by the 31–45 age group, comprising 27%, and the 46–60 age group, which represented 16%. The lowest occurrence was recorded in the 61–80 age group, accounting for only 5% cases of the total. These findings indicate that nephrolithiasis is most common among individuals in their middle age, particularly those between 16 and 45 years, which may be attributed to higher occupational stress, dietary habits, reduced fluid intake, and metabolic changes that typically emerge during this period of life.

The quantitative analysis of renal calculi using a UV-Vis double beam spectrophotometer revealed the presence of calcium (Ca) in all stone specimens. Calcium was the most abundant, consistent with predominance of Ca-based calculi in study populace. The highest calcium content was observed in calcium oxalate (CaOx) stones, with an average concentration of 33.4 mg/g. Mixed stones (COM + uric acid or COM + struvite) contained significantly lower calcium levels 8.7 mg/g, while uric acid and struvite stones had average calcium concentrations of 4.16 mg/g and 6.74 mg/g, respectively.

Table 1. Calcium Concentrations in different types of Renal Stones

Stone Type	Calcium (Ca, mg/g)
Calcium Oxalate (CaOx)	33.4
Mixed (COM + UA/Struvite)	8.7
Uric Acid (UA)	4.16
Struvite (Str)	6.74

The Fourier Transform Infrared (FTIR) spectroscopic analysis of renal calculi collected from 100 patients revealed distinct absorption bands corresponding to various crystalline and molecular components. The FTIR spectra confirmed that majority of calculi were Ca-based calculi, with calcium oxalate monohydrate (COM; whewellite) identified as the predominant constituent.

Calcium oxalate monohydrate (COM) was detected in 48 cases (48%), characterized by strong absorption peaks around 1620–1630 cm^{-1} and 1310–1320 cm^{-1}, corresponding to C=O stretching and C–O bending vibrations of oxalate ions, along with a broad band near 3450 cm^{-1} due to O–H stretching of water molecules. Mixed stones containing COM and uric acid were observed in 8 cases. Their spectra exhibited combined features of both constituents, including oxalate bands (1315–1620 cm^{-1}) and characteristic uric acid peaks near 1680 cm^{-1} and 750 cm^{-1}, indicating the coexistence of these two crystalline phases.

Calcium oxalate–struvite mixed stones, found in 4 cases, displayed the typical COM absorption peaks along with additional phosphate and ammonium bands of struvite, notably at 1010–1030 cm^{-1} (P–O stretching) and 1430–1450 cm^{-1} (NH_4^+ deformation). Pure uric acid stones were identified in 19 patients (19%), exhibiting intense absorption bands at 1680 cm^{-1} (C=O stretching) and 750–800 cm^{-1} (N–H bending), confirming their uric acid composition. Struvite stones, present in 13 cases, showed diagnostic peaks at 1010–1030 cm^{-1} for phosphate and 1430 cm^{-1} for ammonium ions, consistent with magnesium ammonium phosphate hexahydrate. CaP calculi were identified in 4 sufferers, characterized by bands near 1035cm^{-1} (P-O stretch) and 603 cm^{-1} (P-O bending). Cystine calculi recognized in 4 patients, characterized by bands near 3026 cm^{-1} (N-H stretching) and 1585 cm^{-1} (N-H bending), as shown in Table 2.

Table 2. FTIR Spectroscopic Analysis of Renal Calculi

Stone Type	No. of Cases (n= 100)	Percentage (%)	Characteristic FTIR Absorption Bands (cm^{-1})	Major Identified Components
Calcium Oxalate Monohydrate (COM / Whewellite)	48	48	3450 (O–H stretch), 1620–1630 (C=O stretch), 1310–1320 (C–O bend)	Calcium oxalate monohydrate
Mixed (COM + Uric Acid)	8	8	1620–1630 (C=O, oxalate), 1315 (C–O), 1680 & 750 (C=O and N–H, uric acid)	Calcium oxalate + uric acid
Mixed (COM + Struvite)	4	4	1620 (C=O, oxalate), 1030 (P–O stretch), 1430–1450 (NH_4^+ deformation)	Calcium oxalate + magnesium ammonium phosphate
Uric Acid (UA)	19	19	1680 (C=O stretch), 750–800 (N–H bend)	Uric acid
Struvite (Magnesium Ammonium Phosphate)	13	13	1010–1030 (P–O stretch), 1430 (NH_4^+ bend)	Magnesium ammonium phosphate hexahydrate
CaP	4	4	1035(P–O stretching), 603 (P-O bending)	Calcium Phosphate
Cystine	4	4	3026 (NH stretching) 2962 (CH stretching) 1585 (NH bending)	Cystine

CONCLUSION

In this study, the results with UV-Vis spectrophotometer showed that CaOx stones were predominantly calcium-rich. Overall, the UV-Vis spectrophotometric data corroborated the compositional analysis, confirming that calcium was the principal constituent in the majority of renal calculi. The examination of renal calculi with FTIR confirmed that the majority of the calculi were Ca-based calculi, with calcium oxalate monohydrate (COM; whewellite) identified as the predominant constituent.

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