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# COMPARATIVE ANALYSIS OF KIDNEY STONES COLLECTED FROM MALE AND FEMALE SAMPLES OF DIFFERENT AGE GROUPS FROM QUETTA CITY

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**Abstract**

***Background:*** *Kidney stone or Nephrolithiasis is a hard crystalline mineral. An infection with a multifactorial etiopathogenesis, kidney stone sickness might very well be caused by calcium stones, uric acid, struvite, or cysteine. Problems with crystallization promoters and inhibitors, or with stone metabolism and excretion, are the cause of most stones. Numerous risk factors, including as dietary practices, exposure to warm weather, and family history, have been demonstrated in epidemiological and clinical research to contribute to the development of illness and can only be diagnose by knowing its compositions. The aim of this study was to identify the kind and chemical composition of kidney stones in different age and gender of our community to guesstimate its relay on age and gender.*

***Methods:*** *The current research has done to examine the chemical type and composition of kidney stones in different age group which ranges from 20 to 46 plus years of different gender. A total of 13 kidney stone samples of males and females (7 samples were of male and 6 were of female) were collected to analyze for chemical composition by FTIR.*

***Results:*** *Only one sample had magnesium ammonium phosphate (Struvite) while seven (7) samples were consisted of calcium oxalate + uric acid+ magnesium ammonium phosphate and the remaining five (5) were observed to contain calcium oxalate+ magnesium ammonium phosphate.*

***Conclusion:*** *Gender wise contrast exposed that majority of those stones were collected from male patients as compared to females, as the observed ratio was (53.84%) and (46.14%) respectively. Age wise distribution showed that ages of patient ranged from 21 to 31 years, which may be attributed to the fact that older stone formers excrete less urinary calcium than their younger counterparts and may show defects in urinary inhibitors of crystallization.*

**Keywords:** *Cysteine, Etiopathogenesis, FTIR, Kidney stone, Nephrolithiasis Uric acid, Urinary calcium*

## INTRODUCTION

Kidney stone, a hard object, is formed by the crystals which segregate from the urine within the urinary tract. It is also known as nephrolithiasis, which is a commonly used medical term. Normally, urine contains chemicals that stop the crystals from developing.

The Kidney stones constituted an organic matrix, majorly including proteins, lipids, carbohydrates, biominerals and cellular components. These inhibitors do not seem to work for everyone, however, so some people form stones.

Kidney Stone Disease (KSD) is one of the most common kidney diseases in the world. It's also one of the costliest health care conditions affecting the quality of life of the world's population. Nephrolithiasis (a type of kidney disease) is on the rise, with an estimated 14% of people in England suffering from it and 10.1 percent in the US (1, 2). If left untreated, kidney stones can block the urinary ducts, release blood into the urine stream, lead to UTIs, vomiting, or painful urination leading to permanent kidney damage (3). Urologic disease is one of the most common and long-lasting diseases in the world. About 50% of people with urologic disease will get it at least once in their lifetime, and about 75% will get it within the next 20 years. The incidence of urologic disease has been on the rise around the world in recent years. Several studies have suggested that kidney stone formation is on the rise due to a variety of environmental factors, such as lifestyle changes and changes in diet. Global warming has also contributed to an increase in the incidence of

kidney stones (4). It is still unknown what specific factors are causing urolithiasis to become more common and to recur. Kidney stone disease has become a public health concern due to its high prevalence in working-age adults. It significantly affects both individuals and society, especially those living in hot, dry climates (5).

Based on the mineralogical composition, there are five primary types of kidney stones: 1.7% Brushite, 12.4% Urate, 65.9% CaOx, 15.6% Carbapatite, and 2.7% struvite. The two main types of kidney stones are non-calcareous and calcareous, or containing calcium. The most prevalent forms of kidney stones in humans are calcareous, radio-opaque stones called CaOx and calcium phosphate (CaP), either isolated or in combination. Kidney stones originate from a CaP base known as Randall's plaques (RPs), which start at the renal papillary surface's cellar membranes on the thin branches of the loop of Henle of nephron. Males are more probable than females to have stones of urate & Ca Oxalate, while females are more expected having stones of struvite & carbapatite. It is still uncertain, nevertheless, how sex differences affect the pathophysiological mechanisms underlying urinary pebble illness. Kidney stone formation, regardless of type, is a multifaceted and intricate process that involves crystal nucleation, growth, and aggregation in the urine (6, 7).

Systemic disorders such as diabetes, obesity, cardiovascular diseases, hypertension, and metabolic syndrome are linked to the formation of kidney stones. On the other side, one with nephrolithiasis, also stated to as KSF (Kidney Stone Formers), are susceptible to the onset of hypertension, chronic kidney disease, hypertension & the advancement of end stage of renal sickness (8-11)

Fourier Transform Infrared spectroscopy is an appropriate method to analyze the structure and configuration of kidney stones with exactitude and rapid results to be achieved rapidly (12). Additionally, presence of the quantitative analysis of the comparative amount of each fundamental is conceivable without using any flush. Consequently, FTIR's is considered to be the most suitable technique for analysis of renal pebble (3).

The study on the composition of kidney stone can supply best summarization of the stone morphology and can provide information about different lithogenic process in the same type of stone and specific metabolic disorders. Stone analysis is a vital part in the estimation of patients having this disease. By knowing the stone composition, the physician could orientate toward specific etiologies and can help the patient to take healthy diet and prevent its formation (13).

In the current study, samples were collected to control the quick valuation of kidney stones by FTIR without by means of any poisonous substance for absorption or abstraction.

# MATERIALS AND METHODS

## SAMPLE COLLECTION

Kidney stone samples were collected from four renowned hospitals of Quetta city which includes Kidney center, Combined Military Hospital (CMH), Frontier Corps (FC) Hospital and Bolan Medical Complex (BMC).

## SAMPLE SIZE

To isolate and identify *S. aureus*, a series of distinct biochemical tests were conducted at microbiology lab, Bahawalpur Victoria Hospital (BVH). These tests served the purpose of differentiating *S. aureus* strains from other staphylococci, such as *S. epidermidis*, and included the following assessments.

## SAMPLE PREPARATION

Morphological features of each kidney stone were observed and then a well homogenous powder was gained, which was later kept in a tube containing silica gel, in shadowy until further analyzed by FT-IR.

## SAMPLE ANALYSIS

A minor quantity of sample (around 1 mg) was placed on the absorbance container in forward-facing of the IR beam. The observed spectra were the middle infra-red range of near 400 $cm^{-1}$-4000 $cm^{-1}$.

# RESULTS

## GENDER BASE ANALYSIS OF KIDNEY STONE

Total 13 kidney stones were collected out of which 7 stones were of males while the rest were collected from female patients (Table I).

**Table I.** Gender wise comparison of kidney stones

| Gender | No. of Stones | Percentage |
|---|---|---|
| Male | 7 | 53.84% |
| Female | 6 | 46.14% |

## AGE WISE COMPARISON OF KIDNEY STONES

The samples were collected from different age group of male and female (Table II).

**Table II.** Comparison of kidney stones (age wise)

| Age ( years) | Number of patients | Percentage |
|---|---|---|
| 20 – 25 | 03 | 23.07% |
| 26-30 | 05 | 38.46% |
| 31-35 | 03 | 23.07% |
| 36-40 | 00 | 0% |
| 41-45 | 01 | 7.69% |
| 46-above | 01 | 7.69% |

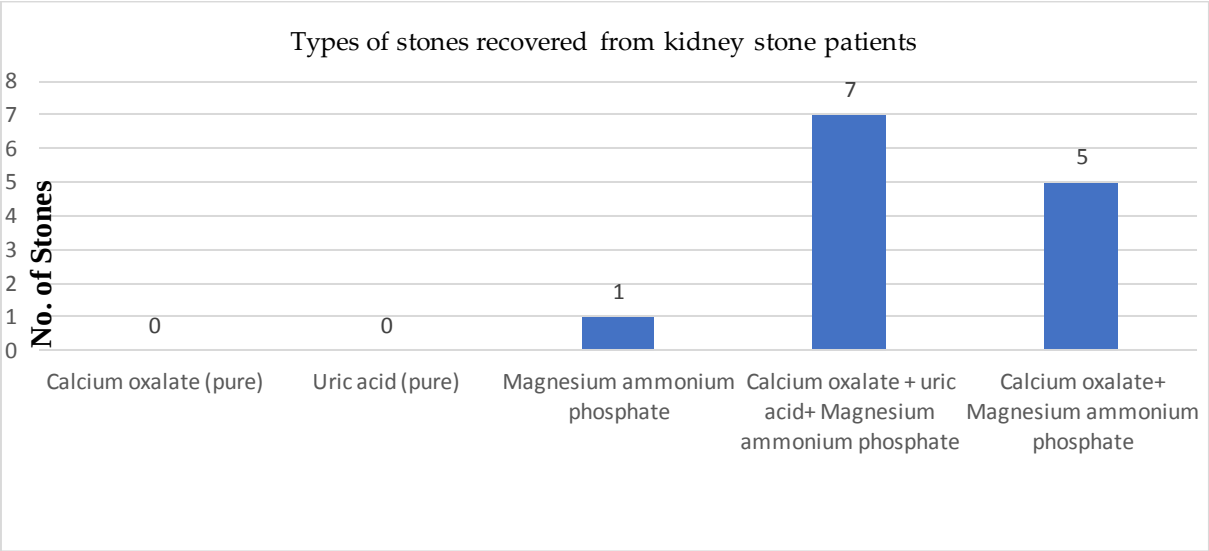


**Fig. 1.** Types of stones recovered from patients

The Fig. 1 characterizes the kidney stones and their frequency of incidence. Out of 13 samples only one sample was pure magnesium ammonium phosphate (7.69%), whereas seven samples were found to be composed of mixture of calcium oxalate + magnesium ammonium phosphate and Uric acid (53.84%). Rests of the five samples were composed of the mixture of calcium oxalate+ Magnesium ammonium phosphate (38.46%).

The FTIR spectra of sample 1 of Kidney stone, revealed the presence of Thioethers (649 $cm^{-1}$), $CH_3S$ (C-S stretch), Aliphatic organohalogen compound group frequencies such as C-Cl stretch of Aliphatic chloro compounds (700-800$cm^{-1}$), C-Br stretch (700–600$cm^{-1}$) of Aliphatic bromo compounds, C-N stretch of aromatic amines, strong S=O stretch sulfone group, and organic phosphates (P=O stretch) and other compounds. In functional group region different peak values were observed which indicates the mixture of calcium oxalate (780.46$cm^{-1}$ and 1315.38$cm^{-1}$) and Struvite ( 2357.05$cm^{-1}$, 1457.29$cm^{-1}$).

The refractive peaks in this sample showed the presence of aliphatic iodo compounds (500-600 cm-1), C-Br stretch (700–600 cm-1) of aliphatic bromo compounds, C=C bending of di substituted (cis) alkene, and medium C-H bending of methyl group (1457.01 cm-1). Surprisingly sample 6 was found to be pure magnesium ammonium phosphate or struvite with a peak value of (2358.79 cm-1 and 1473.26 cm-1).

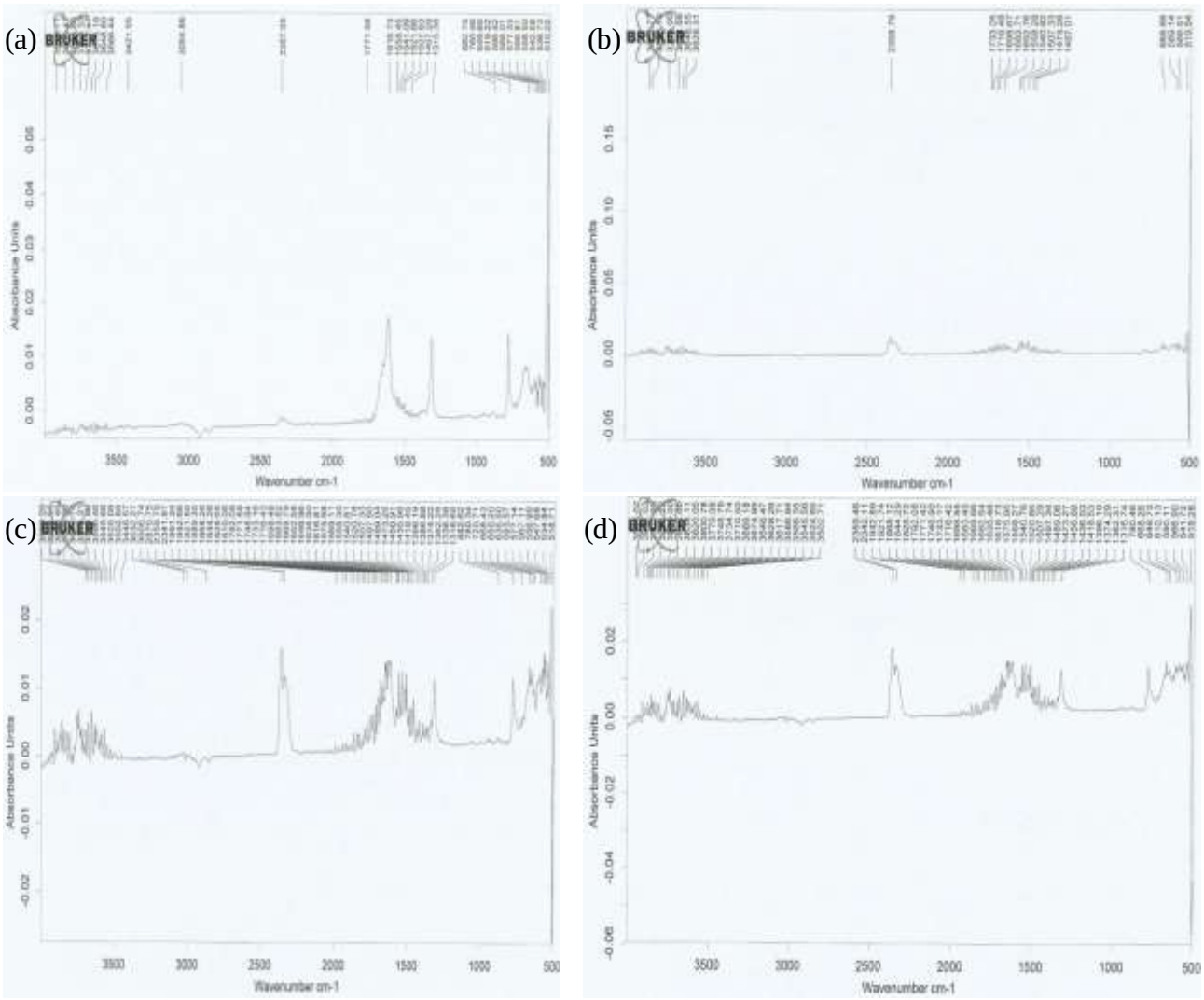


**Fig. 2 (a).** Sample 1 male patient (Age 41), **(b)**. Sample 6 female patient (age 25), **(c).** Sample 7 male patient (Age 23), **(d).** Sample 8 female patient ( Age 24)

The spectral observations in this sample revealed that it consists of aliphatic iodo compounds 500-600 $cm^{-1}$, C-Br stretches near 700–600 $cm^{-1}$ , C-Cl stretching frequency was 700-800 $cm^{-1}$, organic phosphate group (P=O)(1338.38 $cm^{-1}$), nitro compounds (1362.25 $cm^{-1}$), C-H bending of aldehydes, (1374.22 $cm^{-1}$), strong S=O stretch of sulfates, literary the sample was composed of mixture of 3 components such as calcium oxalate ( 780.34 $cm^{-1}$,1316.65 $cm^{-1}$, 1616.81 $cm^{-1}$) magnesium ammonium phosphate ( 2359.75 $cm^{-1}$, 1473.25 $cm^{-1}$) and Uric acid ( 1635.50 $cm^{-1}$, 720.71 $cm^{-1}$).

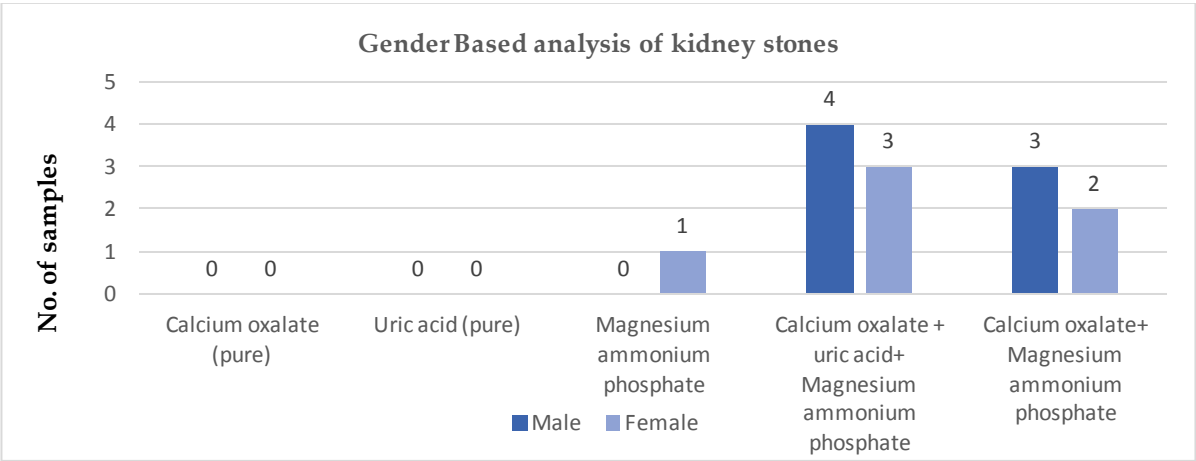


**Fig. 3.** Gender based analysis of kidney stones

The FTIR spectral observations in this sample showed that it consists of Aliphatic iodo compounds (500-600 $cm^{-1}$), C-Br stretch (700–600 $cm^{-1}$ ), disulfide stretch (C-S) (635.50 $cm^{-1}$, 650.20 $cm^{-1}$ and 668.43 $cm^{-1}$),

organic phosphate group (P=O)(1338.38 cm-1), nitro compounds (1362.31 $cm^{-1}$), C-H bending of aldehydes, (1374.22 $cm^{-1}$), strong S=O stretch of sulfates, strong O-H bending of phenol (1418.53 $cm^{-1}$). The sample was mainly composed of mixture of calcium oxalate, uric acid, and magnesium ammonium phosphate with peak values of ( 780.46 $cm^{-1}$, 1316.74 $cm^{-1}$), (1635.48 $cm^{-1}$), (2359.46 $cm^{-1}$, 1473.27 $cm^{-1}$) respectively.

In Fig.3, it was evidently found that the ratio of kidney stones from the mixture of calcium oxalate, uric acid and ammonium phosphate was higher in males as compared to females.

**Table III.** Kind of stones, presence and IR bands of principle constituents observed in kidney stones

| Kind of stones | Presence in Kidney Stones (n=13) | Principle IR Bands observed in current work | IR bands Detected in literature & standards |
|---|---|---|---|
| Pure Calcium oxalate | 0 | Nil | C=O asymmetrical Stretches 778.53, C-C symmetrical stretching, 1314.93, O-C=O asymmetrical stretching 1604.64 |
| Pure uric acid | 0 | Nil | C=C stretching 1637.29, N-H stretches 1018.13, C-N stretches due to aromatic 738.03. |
| Struvite | 1 | 2358.79, 1473.26 | C-H and N-H Stretching on 2362.63, 1469.19 $NH_3$ & symmetrical bending, 970.53 on P-O-C aliphatic Stretches |
| Uric acid + CaOx + Magnesium ammonium phosphate | 7 | 779.74, 1314.92, 1616.51, 1635.48.2359.47, 1473.26. | CaOx, uric acid & Magnesium ammonium phosphate |
| Calcium oxalate+Struvite | 5 | 779.74, 1314.92, 1616.51, 2359.47, 1473.26. | CaOx & Magnesium ammonium phosphate |

The spectral peaks recognized for calcium oxalate bands near 1315.62, 778.96, 1616.51 $cm^{-1}$, uric acid 1635.48 $cm^{-1}$, magnesium ammonium phosphate 2359.79, 1473.48 $cm^{-1}$, and for magnesium ammonium phosphate and calcium oxalate was near 779.74, 778.93, 1314.92, 1616.51, 2359.54, 1473.24 respectively (14, 10).

# DISCUSSION

The kidneys perform a number of tasks, such as excretion, water and acid-base regulation, electrolyte balance, drug detoxification, hormone regulation, and blood regulation. For the 1950s, renal stones, or nephrolithiasis, were recognized. Kidney stones can be of four different types: calcium, cysteine, uric acid and struvite.

Based on an analysis of kidney stone incidence by gender and age, the current study found that males had a higher incidence of kidney stones (53.84%) than females (46.14%). Male kidney stone incidence has been linked to higher dietary protein intake, which lowers urinary citrate concentration and increases phosphate and magnesium excretion in the urine. Because women's urine contains fewer salts that can form kidney stones, there is a lower risk of kidney stones forming. This has been linked to an increase in urine citrate concentration as observed by John *et al*., 2021 (15).

The indicative bands are approximately 3000 $cm^{-1}$ of OH Stretch, 1616 cm-1, 1314 cm-1 of C=O and C-O Stretch, 1473 cm-1 of NH4, and 780 cm-1 of phosphate for calcium oxalate (mono/dihydrate) in mixture with magnesium ammonium phosphate & calcium phosphate pebbles. It can be easily observed even in mixed salt stones by looking for other bands at 2377 cm-1, 1476 cm-1, 1437 cm-1, 872 cm-1, 770 cm-1, and 574 cm-1 in addition to the strong absorption band at 1280–1350 cm-, which is caused by the absorption of phosphate group. The group vibration is responsible for the bands at 1476 cm-1 and 1437 cm-1. When struvite and apatite are associated, the latter's presence can be deduced by absorption at 600 cm-1cm-1 and a

shift of the band toward higher frequencies at about 1010 cm-1 as approximately same results shown by Sofińska-Chmiel *et al.,* 2023 (16) .

The age range for kidney stone disease presentations, according to age distribution, was 21 to 30 years old. This may be explained by the fact that older patients excrete less calcium in their urine than younger patients do, and they may also have problems with their urinary inhibition of crystal growth (17).

The diagnostic bands for samples of magnesium ammonium phosphate stones are approximately 2359 to 2362 cm-1 (N-H and C-H Stretch) and 1469 cm-1 to 1475 cm-1 (NH3 symmetric bending). The current study's results were fairly similar to those of the study conducted by [15] because one of the samples was struvite (pure magnesium ammonium phosphate).

# CONCLUSION

Thirteen kidney stone samples were processed in order to use FTIR to analyze the chemical composition of the male and female samples. Seven samples consisted of calcium oxalate + uric acid + magnesium ammonium phosphate, and the remaining five samples were observed to contain calcium oxalate + magnesium ammonium phosphate. Only one sample contained magnesium ammonium phosphate (Struvite). It was evident that none of the thirteen samples contained pure uric acid or pure calcium oxalate. Gender-based comparative analysis revealed that male patients accounted for 53.84% of the total stones obtained, while female patients accounted for 46.14%. The age group with kidney stone disease ranged from 21 to 30 years old, according to the age distribution. This might be explained by the fact that older age people excrete less calcium in their urine than younger ones. It might have issues with the crystallization inhibitors in the urine. An efficient and accurate method for figuring out kidney stone composition is FTIR.

## Recommendations:

Based on current research and a review of the literature, it is strongly advised that people increase their daily water intake as this will definitely lower their risk of kidney stones. Individuals who drink less water are more likely to develop multiple kidney stones. Controlled dietary intake, strict avoidance of foods that promote kidney stone formation and regular urination are all important because skipping a pee session can increase the risk of kidney stones developing.

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