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Original Article

Frequency and Spectroscopy of Renal Stones on Perkin Elmer FTIR Spectrum 2 Instrument

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ABSTRACT

Renal stones are solid masses, which are a build-up of crystals. They usually develop anywhere in urinary system consisting kidneys, ureters bladder and urethra. Renal stones cause severe pain in the respective organ which may vary according to stone size and shape. Their etiology depends on geographic, socioeconomic and clinical factors. **Objective:** To determine the frequency and spectroscopy of renal stones on Perkin Elmer FTIR Spectrum Two Instrument. M**ethods:** A study was conducted on200 patients, both males and females with renal stones having age groups of 1-20, 21-40 and 41-60 years for the duration of 11 months in Lahore, Pakistan. Demography on risk factors for stones included family history, obesity, gastro-intestinal disease, surgery, dehydration and certain diets was observed. A focus was on number of stones, its size, colour, shape and surface build up. The spectroscopy of stones was carried out using Perkin Elmer FTIR spectrum 2. **Results:** Renal stones were observed to be more frequent in males than females. Meanwhile, females have common features of renal stones but may have variations in some properties. Age group 21-40 years have high frequency with 143 patients (36.67%). In spectroscopy nature and shape of stone reveals that most common shape of the stone was irregular and 68% were pure stones while 33% mixed in composition and properties. **Conclusions**: The formations of stones may depend on geographic, socioeconomic and clinical factors with varying frequencies and properties in male and female ratio.

INTRODUCTION

Renal stones are hard aggregates that form with in kidneys. They develop crystals which really congregate together around the inner surfaces of the kidneys. Smaller stones sometimes used flow through the urinary tract and gradually knock out of the body with urine without being detected [1]. It is the most common disease that affects men between the ages of 30 and 60 [2]. Renal stone is a chief source of morbidity and influences about 1–15% of the population globally. [3] Renal stone patients are more likely to experience chronic kidney disease (CKD), end-stage renal disease (ESRD), and cardiovascular diseases [4]. Fascinatingly the prevalence of Renal Stones has increased, considered partially owing to affluence rather than poverty [5]. Most of the renal calculi are composed of calcium oxalate about 65% to 70%, calcium phosphate 16% to 20%. However, it is believed that the risk of nephrolithiasis is increased with a high intake of calcium, there is promising evidence that the risk of renal calculi may decrease by 35% to 50% with higher dietary calcium [6]. In history, calcium oxalate has been the most common renal stones, but in contrast new studies from China and Germany have proposed that other subtypes of stones may be more common than calcium oxalate and that stone composition may be varying over time. Moreover, it

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becomes visible that the composition of stones may be altering over time in some nations [7]. In Pakistan uric acid stone occurrence was 28% and 22 % in Israel, whereas only 8–10 % of all renal stones were reported in US [8].
The systemic and hereditary diseases are related to calcium renalstones; most such stones are idiopathic and have as a minimum of one metabolic abnormality. It is diagnosed by 24-hour urine testing [9]. In obese patients, uric acid stones and calcium oxalate stones are most commonly observed repeatedly[10]. Renal stones are often identified by the use of more radiographic imaging techniques, and their clinical importance in this setting is unidentified [11]. Annual prevalence is 3-5% and life time prevalence is 5-25% approximately. In the majority of the renal stones patients, nephrolithiasis is liable to be recurrent. Approximate recurrence rates of renal calculi are 10%, 50% over a period of 5-10 years and 75% over a period of 20 years. However, the incidence rates of nephrolithiasis differ with the terrestrial region of a country [12].The prevalence of renal calculi differs with respect to the age with low prevalence in childhood and the elderly and high in the fourth to sixth decades of life. There is a significant aspect to reflect on associated with the age is that prevalence and incidence of renal calculi are two different entities. There has been a constant male preponderance in prevalence of renal calculi over a century [13].
According to the National Health and Nutrition Examination Survey (NHANES), stone condition affects 6.3 percent of males and 4.1 percent of females.[14]Renal stones affect 3% to 10% of the population, compared to one in every ten thousand males in the total population [15].Over the last three decades, the prevalence of nephrolithiasis has increased in the United States. This increase has been observed in most European countries along with Southeast Asia.[16]. In Western countries, the prevalence ranges from 2 to 19 percent, with Men having a higher incidence. Renal stone prevalence in Taiwan was reported to be 9.6% (14.5 percent in men and 4.3 percent in women) in a previous study [17]. Renal stones affect 5-15 percent of the world's population. Recurrence rates are approaching 50%, and the cost to individuals and society is significant. [18].The burden of CKD is rising in developing nations, notably Pakistan, and is deteriorating due to a lack of community awareness, an abnormally high incidence of established CKD risk factors, and pitiful access to renal replacement therapy. Thus according recent population-based studies from Bhopal, India, there have been 150 incidences of end-stage renal disease per million people [19]. Renal stone disease is a common condition in the United States, affecting roughly 1 in 11 people at some point in their lives. Stones are also likely to recur, with at least half of those who have had one having another within ten years of the first [20,21].

METHODS

Data was randomly collected in 11 months about the frequency of renal stones from the inhabitants of Lahore city. Patients with renal stones were clinically examined on ultrasound. Data on the epidemiology of the disease including number of stones, shapes, colour, surface and chemical composition of the renal calculi were collected with the help of Perkin Elmer FTIR spectrum 2.390Samples from patients of both genders of different age groups of 1-20, 21-40 and 41-60 years were collected for spectroscopy.

Physical examination of stones:

In physical examination, grossing was done in which number of stones was computed after determining the number of stone and color of stone either it is yellow, brown or grey etc. Morphology of stones was also observed i.e. shape that might be regular, irregular or oval and also its surface that may be smooth or rough. The size of the stones was measured with the regular scale mostly in 'cm'.

Sample preparation:

The stones were housed in sterile plastic containers and assigned a unique identification number. All of the stones were cleaned to remove any blood, cell components, and other impurities. The stones were dried and stored for 24 hours in an air-conditioned environment whilst being analyzed. Smaller stones (just under 12mm) were mixed and grounded, while the larger stones (greater than 12mm) were left intact. The stones were fissured with a fine needle to obtain separate core and surface samples during FT-IR spectroscopy. The stones were then grounded with a pestle and mortar into a fine, homogenous powder. After each specimen, the knife was properly cleaned with a dry tissue and deionized water to avoid contamination of the specimen by the preceding ground stone specimen.

Stone analysis and reporting by FT-IR spectroscopy:

A dry potassium bromide was combined into a 3mg crushed stone with a weight of 297mg. It was then pressed at 7-11t/cm^2 in a suitable dye to form a transparent 300mg pellet. The pellet in the holder was placed in the spectrometer's IR beam. The spectral range studied was 400 to 4000 cm^2 each spectrum was averaged from 32 scans with a resolution of 4 cm^2.The Euclidean, a tool in the SPECTRA NICODOM IR Library (obtained from Nicodom s.r.o, Hlavni 2727 CZ-14100 Praha 4, Czech Republic, EU) that links the unknown spectrum with reference spectra in the library between 400 and 4000 cm^2, was then used to computerize the spectra. Following that, a report for various stone components was created. The results of the

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automatic spectral recognition analysis were provided as a list of best spectra, along with a score. The score might be anything between 0.000 and 1.000. A score of 1.000 implies that the unexplained spectrum and the reference spectrum are inextricably linked. It would double the results; an optical examination of the spectra was performed for each case.

RESULTS

The results in Table 1 shows that age group hit the highest point of the frequency of renal calculus disease observed in 41-60(40.77%) years, followed by 21-40 (36.67%) years and then 1-20 (22.56%) years. The patient of highest age in the study was of 60 years and lowest of 1 year. In the age group of 41-60 years, the maximum number of cases were 159 (40.77%). Afterwards, the age group with high frequency was 21-40 years having number of cases 143 (36.67%). The smallest number of stone formers was present under the age of 20 years. The least frequency was in the age group 1-20 years (Figure 1). The results of detailed analysis of renal stones showed that among the three age groups, the highest number of patients was presented with multiple stones, followed by the patients having only one stone and then the least number of patients with two stones. Concerning about the colour of renal stones, the highest number of stones presented with brown colour, and less number of stones having grey and very rarely yellow colour were observed. The shape of the stone which is in abundance was irregular, followed by longitudinal and then least number of stones presented with oval shape. In accordance with the surface of stones, the rough surface of stones was predominant over smooth surface (Table 2).

Age groups (yrs)	Total	Percentage	Males	Females	M:F Ratio
1-20	88	22.56	63	25	2.52:1
21-40	143	36.67	101	42	2.40:1
41-60	159	40.77	118	41	2.88:1
Total	390	100	282	108	2.61:1

Table 1: Frequency of age groups and gender

Number of stones	1-20 years; %	21-40 years; %	41-60 years; %
Multiple	51.42	48.75	46.15
One	28.57	41.25	32.69
Two	20	10	21.15
Colour			
Brown	94.28	98.75	94.23
Grey	5.71	1.25	2.88
Yellow	0	0	2.88
Shape			
Irregular	85.71	93.75	92.30
Longitudinal	9.25	3.75	4.49
Oval	5.04	2.5	3.21
Surface			
Rough	99	98	98.20
Smooth	1	2	1.8

Table 2: Number, Colour, Shape, and Surface of Renal Stones

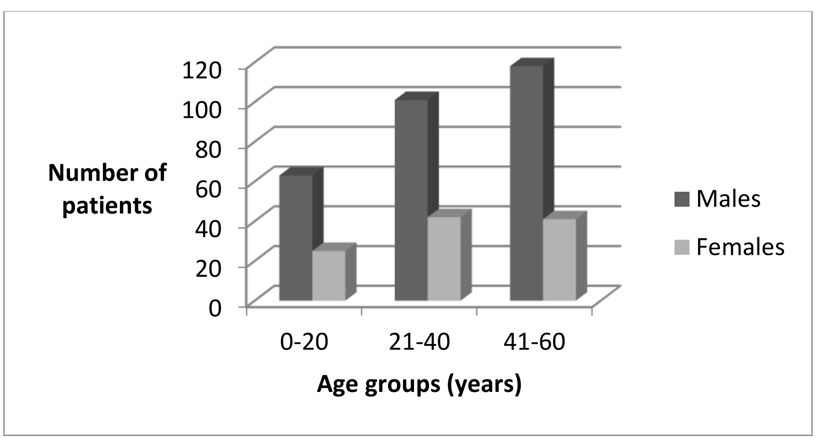

Figure 1: Number, Colour, Shape, and Surface of Renal Stones groups

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The findings of this study reveal the nature, colour, shape and surface of stones. In which, the pure stones were 68% while 33% mixed in traits. The stones examined reveal a total 46% calcium oxalate and 14% uric acid. Only 5%and 3% percentage of ammonium urate and cholesterol stones were analysed. Three percent of all the stones were mixed; combinations were CaOx and Uric acid, CaOx and carbonate apatite, CaOx and ammonium urate and some others (Figure 2).

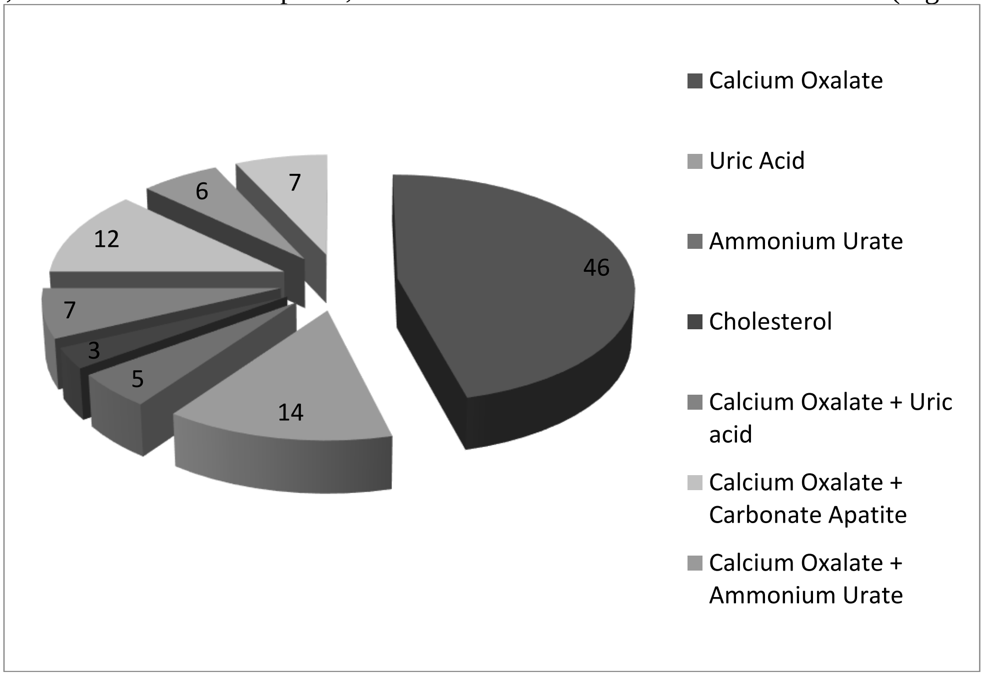

Figure 2: Prevalence of Nature of Renal Stones

DISCUSSION

Renal Stones most common urological disease in Pakistan[1]. In current study the frequency of renal stones was determined with respect to age and gender. The chemical composition of renal stones collected from the patients was also determined on spectroscopy. An age specific frequency was studied and the findings were related to the results of Ahmed et al., who pointed the highest prevalence in the age group of 30-50 years. Gender specifically the overall prevalence is higher in males than females. In our analysis, the male to female ratio was 2.61:1, which is extremely close to the 2.8:1 found in the reported cases in Southern Punjab, Pakistan. The influence of sex hormones on numerous lithogenic risk factors may explain the greater frequency of renal stones in males in this study. Androgens are found to increase oxalate excretion and calcium oxalate crystal deposition in the kidneys, both of which are significant risk factors for the formation of renal stones, whereas estrogen lowers urinary oxalate excretion. Males are more likely to get renal disorders. It's also believed that this is attributable to the fact that males have more muscular mass than females. As a result of the daily breakdown of tissue, the quantity of metabolic waste increased with an increase, raising the chances of getting a renal calculus. Another significant element is that males' urinary tracts are more complicated than females [1].

These results are also similar to that of Javed et al., who proposed that the highest prevalence is in age 15-45 years [22]. Another study conducted by Akram et al., and they suggested the high prevalence in the age group of 40-60 years[23]. This result is in agreement to that of Scales et al., who reported in their study that the overall prevalence of stone disease is 10.6% in males and 7.1% in females [14]. Calcium oxalate was the predominant renal stone in this study. Of the total stones, 46% renal stones were of pure calcium oxalate. This correlates with the work of Akram et al., who found that the calcium oxalate was 58.6% of all the cases [23] renal stones containing calcium oxalate were the most common form identified in the study in Rawalpindi, Pakistan (more than 90%), with pure calcium oxalate (CaOx) calculi contributing to 34% and mixed calcium calculi accounting for 58 percent [24]. Another work from Khyber Pakhtunkhwa Province, Pakistan, reported that calcium oxalate (38%) was the most frequent component of the renal stones [25]. In a study conducted in Larkana, Pakistan calcium oxalate prevailed in 33.1% of stones [26].

Uric acid was also common in most patients comprising 14% pure uric acid stones. The main cause in greater prevalence of uric acid in being overweight, obesity with greater BMI and metabolic diseases [8] in some conditions. Gout is a condition wherein roughly 10-20% patients produce additional uric acid. A purine enriched diet can be associated. A pH of 5.5 is a major risk factor for uric acid stone formation. The essential deficiency appears to be ammonia excretion in the event of

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normal uric acid excretion in urine. The majority of these stones are radiolucent, displaying bright or radio opaque whenever calcium is present.

The dietary habits include excessive acid intake, as high endogenous acid formation defective ammonium execration plays an important role relating other factors for uric acid stone formations. An etiology of renal stones production as mixed type may have several factors differentiating in different population. These risk factors can be caused by excessive intake of oxalate in food causing hyperoxaluria, hypercalciuria, hyperthyroidism, hypocitraturia and renal tubular acidosis [1].

CONCLUSIONS

Males were more likely than females to suffer from renal stone disease in Lahore, Pakistan. The age range 41-60 years had the highest frequency. In Spectroscopy the detailed analysis of renal stones were seen multiple in number, irregular in shape, brown in colour and rough in surface predominantly common. Concerning about chemical composition renal stones were a build-up of pure chemical composition. The most prevalent type was calcium oxalate stone.

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