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Original Article

Assessment of Frequency and Composition of Renal Stones in a Reference Laboratory of Pakistan

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ABSTRACT

Objective: To determine frequency and chemical composition of renal stones in Northern Pakistan using FTIR Spectroscopy.
Study Design: Cross-sectional study
Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jan 2017 to Jul 2020.
Methodology: One thousand and twenty-nine stones were analysed in 3 years at AFIP of patients aged from 1 to 98 years. They were analysed by Automatic FT-IR type IRAffinity-1. The results were verified by manual analysis of spectrograms and then confirmed by standard peaks feed on NICODOM LIBRARY.
Results: Calcium oxalate stones were the most frequent (1065,80.1%), followed by uric acid stones (179,13.5%), mixed stones (44,3.3%), calcium appetite (31,2.3%), struvite (6,0.5%), hydroxy appetite (1,0.1%) and cysteine stones (3,0.2%). Male predominance was noted in patients with renal stones (1057,79.5%). The majority of the patients were from the age group 21 – 40 years (47.5%)
Conclusion: Calcium oxalate stones are most commonly followed by uric acid stones in patients of northern Pakistan. The frequency of renal stones is high in male patients.

Keywords: Calcium oxalate, FT-IR Spectroscopy, Renal stones.

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INTRODUCTION

Nephrolithiasis, one of the most painful diseases, affects 1 in 11 individuals worldwide and causes a significant burden on the health system.[1,2] In Asia, about 1 to 19.1% of the population suffers from nephrolithiasis.[3] Pakistan is located in the "Afro-Asian stone belt", a region reported to have a very high incidence of renal stones. In Pakistan incidence of nephrolithiasis ranges from 7.4 in the north to 200 per 100,000 in the south.[4]

The lifetime incidence of recurrent renal stones ranges from 10– 75%, leading to end-stage renal failure in 2–3% of these patients.[5] Urological interventions have resulted in a remarkable decrease in CKD cases due to recurrent nephrolithiasis.[6] However, this reversible cause still accounts for 8-10% of CKD cases in Pakistan due to late treatment.[6,7] Secondly, analysing the renal stone for chemical composition plays a pivotal role in treating underlying causes and preventing its recurrence, which is also a neglected step in our part of the world.[8]

Fourier transform infrared spectroscopy (FT-IR) is considered a reference method for stone analysis.[9] Furthermore, this technique is preferred due to fast detection and less specimen requirement.[10] Therefore our study aimed to determine the frequency of renal stones of various chemical compositions in our setup to treat and prevent their recurrence.

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METHODOLOGY

The cross-sectional study was carried out at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology Rawalpindi Pakistan after approval from the Institutional Review Board (Cons-CHP-5/READ-IRB/21/150). Data were retrieved from laboratory software from January 2017 to July 2020. Non-probability convenient sampling technique was used. Sample size was using WHO calculator, by taking 7% prevalence.[7]

Inclusion Criteria: The study included patients of either gender and of all age groups with renal stones. One thousand three hundred twenty-nine patients with renal stones were inducted in the study, whose complete data was available in the laboratory software.

Exclusion Criteria: Patients with incomplete data were excluded from the study.

Data was kept confidential. Patients' names and identity was replaced by unique code. Stone samples were received in sterile containers, cleaned with deionised water, dried, and stored in the air-conditioned

environment at 20°C before analysis. Stones having a size greater than 12mm were fractured with the help of a sharp needle to get to the core of the stone. They were then crushed and converted into fine, homogenous powder with the help of a pestle and mortar. The pastel and mortar, after every use, were cleaned with distilled water and dried to prevent contamination by the former stone ground. Stones were then analysed by FT-IR spectroscopy.

Spectrum's background was initially measured with the attenuated total reflectance (ATR) without any sample. Afterwards, the sample holder was cleaned with the help of an alcohol swab. Around 2mg of powdered sample was placed on the flat surface of the spectrophotometer. Eventually, the computer automatically made the spectrum for the renal stone. The spectra of the renal stones were compared with the best-fitting spectra from the NIDCOM library visually.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as mean±SD and qualitative variables were expressed as frequency and percentages.

RESULTS

Of 1329 stone specimens, 1057(79.5%) were from male patients, and 272(20.5%) were from female patients. Our study population was divided into five groups based on age, as shown in Figure-1. The highest frequency of nephrolithiasis was observed in adults of age 21-40 years (47.6%), followed by 41 to 60 years (31.6%) of age.

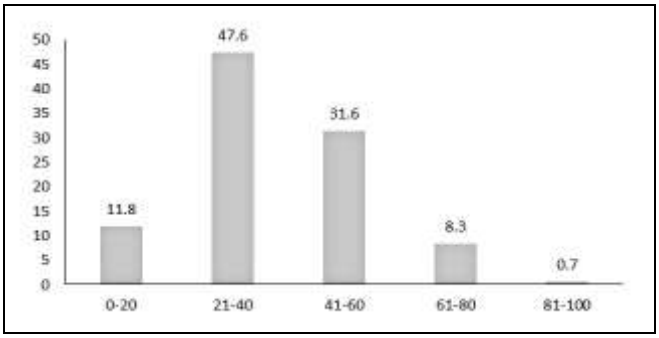

Figure-1: Distribution of Renal Stones in Different Age Groups (n=1329)

The majority of patients, 79.5%(1065), had calcium oxalate stones, while hydroxy appetite stone was found to be 0.1% (1) and cysteine stones were 0.2% (3). Besides these stones, 3.3% (44) patients were found to have mixed stones (Table-I). The composition of mixed stones is shown in Table-II.

Table-I: Composition of Renal Stones by Fourier Transform Infra-Red (FTIR) Spectroscopy (n=1329)

Stones Composition	Number (Percentage)
Calcium oxalate	1065(80.1)
Uric acid	179(13.5)
Calcium phosphate	31(2.3)
Struvite	6(0.5)
Hydroxy apatite	1(0.1)
Cysteine	3(0.2)
Mixed	44(3.3)

Table-II: Composition of Mixed Stones (n=44)

Types of Mixed Stones	n(%)
Calcium oxalate (60-70%) +carbonate apatite (30-40%)	30(68.1)
Calcium oxalate (30-40%) + carbonate apatite (60-70%)	2(4.54)
Calcium oxalate (30-40%) + Uric acid (10-20%) + carbonate apatite (40-60%)	4(9.09)
Calcium oxalate (30-40%) + carbonate apatite (10-20%+ struvite (40-60%)	4(9.09)
Carbonate apatite (40-50%) +struvite (20-30%) + uric acid (30-40%)	3(6.81)
Calcium oxalate + uric acid	1 (2.27)

DISCUSSION

Nephrolithiasis afflicts patients worldwide, requires surgical interventions and hospitalisations, may lead to kidney injury, and increases healthcare costs.[9] Infrared spectroscopy is considered a primary technique to analyse the composition of renal stones.[10] The purpose of this study was to differentiate stones qualitatively. Analysis of stone composition is essential for treating underlying causes and preventing stone recurrence. In our study, male predominance was noted in patients with renal stones. Out of 1329 patients, 1057 patients were males. The male-to-female ratio was 3.8:1. This is similar to previous studies conducted in Islamabad,[11] and Karachi,[8] showing ratios of 2.7:1 and 4.6:1, respectively. The complicated urinary tract in males causes over-acidification of urine, causing increased excretion of oxalates and uric acid and suppressing the excretion of citrate, an inhibitor of stone formation.[10]

The highest frequency of stones in our study is in the age group 21 to 40 years, followed by 41 to 60 years. Joshi *et al*. also reported the highest renal stone prevalence in the same age group in their study in Nepal,[12] as reported by Samad *et al*.[13]

Calcium oxalate stones were found to be most abundant in our results, i.e. 79.5%. In an Indian study, calcium oxalate stones were reported to be 93%14 and

86% in Nepal.[12] It has been postulated that the dramatic changes in dietary habits that have been observed in a couple of last decades, like high protein and salt intake and consumption of carbonated beverages, represent the major causes of the high incidence of calcium oxalate renal stone, which has now become the most frequently diagnosed renal stone.[14] Oxalate is the end product of several metabolic pathways (including those in ascorbate and hydroproline). In addition, 10% to 20% urinary oxalate is derived from dietary sources such as food rich in oxalates like spinach, tea and cranberries. Hence, any condition that increases oxalate absorption from food may lead to increased oxalate production and causes calcium oxalate formation.[15]

Uric Acid stones were the second most common stone in our study (13.5%). A similar study conducted in Multan (Pakistan) by Rafique *et al*. has shown uric acid stones to be 28.1% of all renal stones.[16] Hyperuricosuria results from a high dietary intake of beef, fish and poultry which may lead to stone formation.[15]

The comparison of the chemical composition of renal stones in local,[11,13] and international studies,[17,18] is given in the Tables-III and IV, respectively. Differences observed in the frequency of different chemical compositions of renal stones among different nations may be due to differences in lifestyle, dietary habits and environmental factors.

Table-III: Comparison of results of Chemical Composition of Renal Stones with Pakistani Studies

Chemical Composition of Renal Stones	Present Study n=1329	Bangash *et al.* (Islamabad)[11] n=232	Samad *et al.* (Multan)[13] n=340
Calcium oxalate	80%	38.36%	40%
Uric. Acid	13.5 %	0.43%	6%
Calcium phosphate	2.3%	-	-
Hydroxy appetite	0.1%	-	-
Cystine	0.2%	-	-
Struvite	0.5%	4.31%	-
Calcium oxalate + calcium phosphate	2.40 %	15.9%	30%
Calcium oxalate + calcium phosphate +Uric acid	0.30%	4.7%	-
Calcium oxalate + calcium phosphate + struvite	0.30%	-	-
Calcium oxalate + uric acid	0.075%	31.9%	10%

CONCLUSION

Calcium oxalate stones are the most common type in our study population. Adult patients of 21-40 years of age are most affected. Male predominance has been observed in all age groups.

Table-IV: Comparison of Results of Chemical Composition of Renal Stones with Other Countries

Chemical Composition of Renal Stone	Present Study (n=1329)	Da silver *et al.* Brazil[17] (n=50)	Hareendra *et al.* Srilanka[18] (n=50)
Calcium Oxalate	80%	32%	86%
Uric Acid	13.5 %	10%	-
Calcium Phosphate	2.3%	4%	2%
Hydroxy Appetite	0.1%	2%	-
Cystine	0.2%	4%	-
Struvite	0.5%	22%	2%
Calcium,oxalate+calcium phosphate	2.40%	10%	-
Calcium,oxalate+calcium phosphate+uric acid	0.3%	10%	10%
Calcium,oxalate+calcium phosphate+struvite	0.3%	2%	-
Calcium oxalate+ Uric acid	0.075%	4%	

Conflict of Interest: None.

Author's Contribution

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

AB & MA: Data acquisition, critical review, approval of the final version to be published.

MR & ZHH: Data analysis, data interpretation, drafting the manuscript, approval of the final version to be published.

SIK & HJ: Conception, study design, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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