Original Article

CHEMICAL COMPOSITION SPECTRUM OF URINARY TRACT STONES BY ATTENUATED TOTAL REFLECTION-FOURIER TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

Objective: The objective of this study was to determine the frequency of urinary tract stones of different chemical composition using ATR-FTIR received for analysis

Material and Methods: In this Cross-sectional study with retrospective sampling was conducted from January 2010 to January 2018, data was collected from 2092 patients whose stones were sent for biochemical analysis to the laboratory after removal or by spontaneous expulsion in a sterile container. Stones that were sent in fluid filled containers, with collection devices and embedded in tissues were excluded. Patient's identity was kept confidential by assigning a new code to each stone. Stones were cleaned and washed with distilled water to remove debris like mucous, blood, rubbish and casts. Stones were converted to a homogenous powder by pestle and mortar. Stones were then analyzed by FT-IR spectroscopy on IRAffinity-1. Mean and standard deviation was calculated for all the parametric quantitative variables while frequency with percentages was used for qualitative variables.

Results: Majority of the patients 1495(71.4%) had calcium oxalate stones followed by mixed heterogeneous type 423(20.2%). The age group that showed the highest prevalence of nephrolithiasis was 30-39 years (25.7%) followed by 20-29 years (21.6%) and 40-49 years (17.8%). The lowest cases of nephrolithiasis were in the 90-99 years of age group (0.04%) followed by 80-89 years (0.5%). Male predominance was found in all age groups

Conclusion: This study concluded that the most common chemical composition of stones is calcium oxalate followed by mixed heterogeneous type. Urinary tract stones are more prevalent in adult males.

Key Words: Urinary stones, FTIR spectroscopy, Urinary tract infection.

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INTRODUCTION

Urinary tract stones are one of the most frequently encountered problem in primary care setups. It can present with classical features of renal colic, dysuria and hematuria. While in other cases it may be asymptomatic or can present with vague symptoms like nausea, urinary frequency, urgency, flank pain, abdominal pain, testicular pain, penile pain and difficulty in urinating [1]. Eighty percent of the stones are formed of Calcium with majority being that of calcium oxalate and phosphate. Other types include Urate, Struvite (magnesium ammonium phosphate), Cystine, Cholesterol, Calcite, Calcium carbonate apatite. Patients can present with both pure stone and a mixture of stones [2,3].

About 2-5% of the population suffer from nephrolithiasis in Asia, while the reported frequency in America, Europe and Saudi Arabia is 0.1-0.4%,0.1-0.4%, and 20% respectively [4]. Pakistan sits in the middle of the area called "stone belt" which stretches from Sudan and Egypt through India, Middle East, Pakistan, Thailand, Burma, Philippines and Indonesia. This area has been reported with increased frequency of urinary tract stones. In Pakistan, people of Seraiki belt of Punjab and Multan are among the most effected [5].

Environmental and genetic factors have both been attributed to the formation of urinary tract stones. Climate and diet are the leading causes among the environmental factors; others being a decreased liquid intake, increased consumption of fructose, sodium, animal protein and calcium oxalate. The four basic mechanisms involved in the pathogenesis of lithogenesis are nucleation, retention of initial nucleus in sites of urothelium, crystal growth, and crystal aggregation. Various theories have also been proposed which include the free particle theory, the fixed particle theory and the interstitial apetite plaque (Randall/s plaque theory) [6].

A novel analytical technique used for the specific and quick analysis of urinary tract stones is

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Fourier transform infrared spectroscopy (FTIR). The ATR (Attenuated Total Reflection) module has been accepted as the most convenient for analysis of routine calculi. It correlates well with the semi quantitative biochemical analysis. As compared to the traditional method, FTIR with ATR module takes half the approximate time with considerable consumables and labor savings [7].

As nephrolithiasis is one of the major problems encountered in our setups therefore our study was aimed to find out the frequency of urinary tract stones of different chemical composition in District Rawalpindi using ATR-FTIR.

MATERIAL AND METHODS

This study was a cross-sectional study conducted after the Institutional Review Board's (IRB) approval from January 2010 to January 2018. Data was collected from 2092 patients whose stones were sent for biochemical analysis to the laboratory after removal or by spontaneous expulsion in a sterile container. Stones that were sent in fluid filled containers, with collection devices and embedded in tissues were excluded. Patient's identity was kept confidential by assigning a new code to each stone. Stones were cleaned and washed with distilled water to remove debris like mucous, blood, rubbish and casts. These were then entirely air dried and were stored between 20º-22º C until the analysis. A sharp needle was used to cut large stones that were more than 12mm to reach to the core. Stones were converted to a homogenous powder by pestle and mortar [8]. The knife was washed and cleaned with deionized water and a dry tissue every time to avoid contamination by the last ground stone. Stones were then analyzed by FT-IR spectroscopy on IRAffinity-1 (Shimadzu Corporation).

Initially when there was no sample, the background spectrum was measured with the attenuated total reflectance (ATR) unit. After this the sample holder was cleaned with an alcohol swab. Almost 2mg of stone (powdered) was applied on the plate and different spectra were collected by the Lab Solutions IR software[10] and these were compared with the spectra in the library. Best fitting spectra was selected by visual examination. Statistical Package for Social Sciences (SPSS) version 24 was used for data analysis. Mean and standard deviation was calculated for all the parametric quantitative variables while frequency with percentages was used for qualitative variables.

RESULTS

Out of 2092 patients selected for the study, 1639(78.3%) were males and 453(21.6%) were females. Mean age of all the patients was 36.5± 16.2 years. Our study population was divided into 10 groups on the basis of age as shown in Table-2. The age group which showed the highest prevalence of nephrolithiasis was 30-39 years 539(25.7%) followed by 20-29 years 453(21.6%) and then 40-49 years 374(17.8%). The lowest cases of nephrolithiasis were found in the 90-99 years of age group 01(0.04%) followed by 80-89 years 12(0.5%). Male predominance was found in all age groups.

Majority of the patients 1495(71.4%) had calcium oxalate stones followed by mixed heterogeneous type 423(20.2%). Frequency of urinary tract stones of different chemical composition along with their gender distribution is summarized in Table-3.

Table-1: Specifications of IRAffinity-1 Shimadzu [9].

Parameter	Characteristic
Optical system	Single-beam optics
Interferometer	Michelson interferometer (30º incident angle)
Beam splitter	Germanium-coated KBr
Wavelength range	7,800 to 350 cm^{-1}

Table-2: Age and gender distribution of patients with nephrolithiasis.

Age Group (years)	Total n (%)	Males	Females	Males to Female ratio
1-9	131 (6.26%)	101 (77.09%)	30(22.9%)	3.3:1
10-19	125 (5.9%)	84 (67.2%)	41 (32.8%)	2:1
20-29	453 (21.6%)	343 (75.71%)	110 (24.28%)	3.1:1
30-39	539 (25.7%)	438 (81.26%)	101 (18.73%)	4.3:1
40-49	374 (17.8%)	304 (81.2%)	70 (18.71%)	4.3:1
50-59	249 (11.9%)	186 (74.69%)	63 (25.3%)	2.9:1
60-69	173 (8.2%)	140 (80.92%)	33 (19.07%)	4.2:1
70-79	35 (1.6%)	31 (88.5%)	04 (11.42%)	7.7:1
80-89	12 (0.5%)	11 (91.66%)	01 (8.33%)	11:1
90-99	01 (0.04%)	01 (100%)	00	-
Total	**2092**	**1639 (78.3%)**	**453 (21.65%)**	**3.6:1**

Table-3: Chemical composition of different urinary tract stones.

S#	Stones	Males n (%)	Females n (%)	Total n (%)
1	Calcium oxalate	1179(78.8%)	316(21.1%)	1495 (71.4%)
2	Calcium phosphate	0	02 (100%)	02 (0.09%)
3	Struvite	10 (83.3%)	02 (16.6%)	12 (0.57%)
4	Urate	137 (85.6%)	23 (14.3%)	160 (7.64%)
5	Mixed	313 (73.9%)	110 (26.0%)	423 (20.2%)
	Total	**1639 (78.3%)**	**453 (21.6%)**	**2092 (100%)**

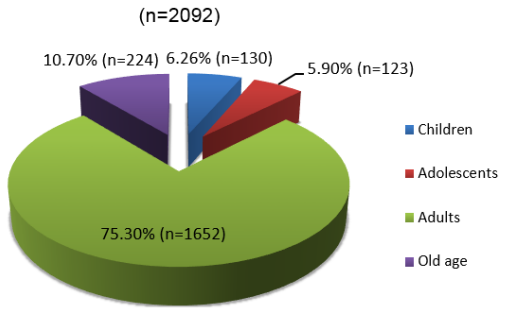

Figure-1: Frequency of renal stones in different age group.

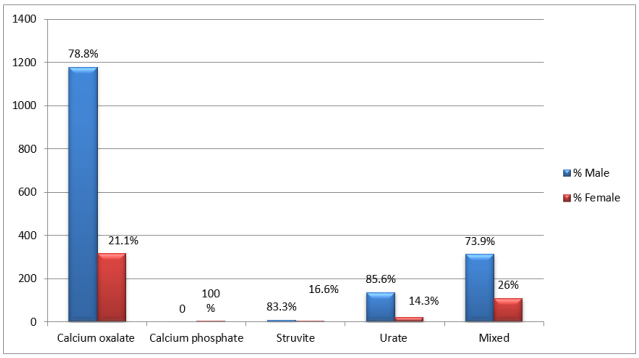

Figure-2: Gender wise frequency of different composition of stones.

DISCUSSION

In our part of the world urinary tract stones are thought to comprise 50% of all the urological cases [11]. Significant morbidity has been associated with it [12]. Urolithiasis can be due to anatomical, metabolic and inherited defects [13,14].

The highest prevalence of urinary tract stones found in our study was between the 3rd and the 4th decade of life which is very similar to that reported by Ahmad *et al* [15]. In another study conducted by Ahmed *et al* [5] in Southern Punjab, the highest prevalence of stones was found in 3-5th decade which is also concurrent with our study. Similar, results of increased frequency of nephrolithiasis cases in the 30-60 years of age group were documented in a study conducted in India with male predominance [16]. Male inclination as found in our study has also been explained by Knoll *et al* [17] in a study conducted in Berlin, Germany. High prevalence in males has also been studied by Scales *et al* [18] in United States.

Calcium oxalate stones which were the most common stones found in our study has also been reported by Bangash *et al* [19] in a study conducted in Islamabad. Highest prevalence of calcium oxalate stones followed by mixed heterogeneous type as reported by our study has been explained by Wrobel et el [20] in Poland. Samad *et al* [21] conducted a study in Multan, showing calcium oxalate stones as the major prevalent stone. Similar results were also given by Hareendra *et al* [22] and Silva *et al* [23] in Sri Lanka. The second most common type of stones as analyzed in our project were of mixed heterogeneous type as explained by Durgawale *et al* [16] in a study conducted at Maharashtra, India.

The ATR module that has been used in our study is a novel technique which has a high signal to noise ratio with reduced scattering. It has the advantage of analyzing a large target area and a high spatial resolution. All of these advantages have been proved by Baker *et al*[10] in a joint study conducted in United Kingdom and Illinois, United States.

Although study reported very important information regarding the diversity of urinary tract stones of different chemical composition in different age groups but being single centered study and with few disadvantages of being the ATR module of FTIR which include destruction due to pressure, spectra interference by air between sample and internal reflection element (IRE) and structural alteration because of IRE and sample interaction[10] were the limitations to our study.

CONCLUSION

Nephrolithiasis is very common in Pakistan like other countries in the stone belt area. Majority of the stones are of calcium oxalate type. Urinary tract stones are more prevalent in 30-39 years of age group with a male predominance in all age groups.

AUTHORS CONTRIBUTION

Asif Ali Memon: Manuscript writing, editing, data analysis

Mehwish Gilani: Sample / Data collection, manuscript writing, data analysis

Ammad Akram: Study design, manuscript editing

Naveed Asif: Sample collection, data interpretation

Nida Basharat: Sample collection

Qurat Ul Ain: Manuscript editing

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