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Research Article

A STUDY OF CHEMICAL COMPOSITION OF RENAL STONES IN HIMACHAL PRADESH

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A B S T R A C T

Introduction: Urolithiasis is a common health problem. Stones develop when there are imbalances of components in urine in conditions like; hypercalciuria, hyperuricosuria, hypocitraturia, low fluid intake, dietary habits like diet rich in sodium, oxalate, fat, protein, sugar, life style and genetic factors. Chemical composition of renal stones is of important as it has bearing on treatment of this ailment. This may help in advising the patients to reduce the incidence and reformation of renal stones. This study was conducted to know the chemical composition of renal stones obtained after surgery from patients at I.G. Medical College at Shimla in H.P. And to find the association of their chemical composition with age, gender distribution, BMI, socioeconomic status and diet in an attempt to understand the etio-pathogenesis of renal stones in this region and compare the same with other regions of the country.

Methods: Renal stones from 50 patients were collected after surgery & chemically analyzed by Fourier transform infrared spectrometry.

Results: Males patients (66 %) were more effected as compared to females (34 %) with high incidence in middle and young population with middle to high socio-economic status, BMI > 25Kg / m^2 and dietary habits such as non-vegetarian diet (66 %). The 62 % stones were of single stone variety and, calcium oxalate was the main constituent, both in male as well as in female population.

Conclusion: Kidney stones were more common in males affecting middle and young population with middle to high socio-economic status, greater BMI and dietary habits such as non- vegetarian diet. The most common stones are of single stone variety and calcium oxalate is the main composition of stones in both male as well as female population.

INTRODUCTION

Stone disease is one of the most widespread diseases of modern society and has been described since antiquity.[1] Medical texts from ancient Mesopotamia, India, China, Greece and Rome all mentioned calculous diseases & humans has been known to be affected by urinary system stones since 4000B.C.[2]

Stone formation may occur when the urinary concentration of crystal-forming substances (calcium, oxalate, uric acid) is high and/or that of substances that inhibit stone formation (citrate) is low, small hard crystals formed when substances like calcium, uric acid, magnesium, ammonium and phosphates precipitate out of urine and build up on the inner surface of kidney.[3]

Changes in lifestyle modifications as less physical activity and change in dietary habits are presumed to lead to increase in the incidence of urolithiasis. Studies from U.S.A shows that 1 person out of 11 persons is having renal stones while in India, 12% population is expected to have urolithiasis during their life span and half of these patients may have deranged renal functions.[4]

Paediatric stone composition and urinary metabolic stone risk parameters are distinct from those of adults, interestingly, girls are more susceptible to nephrolithiasis than boys. It affects all ages, sexes, and races, more common in middle age men, within the age of 20-49 years[5] with life time risk of approximately 13 % in men and 7 % in women. Recurrence is common; hence prophylaxis plays an important role in prevention of urinary stones.

Primary hyperparathyroidism obesity, diabetes and gout, dietary factors like low fluid intake and low dietary calcium increases the risk of nephrolithiasis. However, evidence is mixed for diets with increased animal protein, low dietary magnesium, low dietary potassium, and increased sodium.[6]

The chemical composition of urinary stones is determined by presence of different chemical substances in urine. Stones may have different shapes, sizes & chemical constituents. Depending upon the chemical substances present in stones and on causes, renal stones are classified into five groups .[7]

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In all urinary system stones, calcium oxalate and calcium phosphate salts are more common & in 80% of cases stones occurs in kidneys. Some studies have reported the incidence of various type of stones as pure calcium oxalate, calcium phosphate and a mixture of both as 50 %, 5 % and 45 % respectively, the major chemical component of calcium stones reported has been brushite or hydroxyapatite. In most of renal stones are calcium oxalate stones and occurs as CaOx monohydrate ($CaC_2O_4H_2O$), and CaOx dihydrate ($CaC_2O_42H_2O$), or a combination of both. Combination of these both occurs in about 60 % of stones, while CaOx monohydrate is thermodynamically more stable & observed more frequently than stable CaOx dihydrate.[8]

Many factors responsible for calcium oxalate stones are hyperoxaluria, hypercalciuria, hyperuricosuria, hypomagnesuria, hypocitraturia, and hyper cystinuria. Urinary pH between of 5.0 to 6.5 favors calcium oxalate stones, while whereas pH more than 7.5 promotes calcium phosphate stones.[9] Calcium stones recurrence is more commonly seen as compared to other type of stones.

In 10 - 15 % of renal stone patients, chemical composition seen is struvite or magnesium ammonium phosphate which are also known as triple phosphate stones. These stones commonly occur in patients having chronic urinary tract infections, having urease producing bacteria most commonly Proteus mirabilis while others are Pseudomonas aeruginosa, Klebsiella pneumonia and Enterobacter. Urease splits urea to form ammonia and CO_2, which raises the pH urine more than 7. This causes phosphate to precipitate on insoluble ammonium products, leading to a large stone to form which occupies the calyceal system of kidney giving rise to shape of staghorn which is more common in females.[10]

While uric acid stones or urate stones constitutes about 3-10% of all types of urinary stones. Diets like meat and fish which are rich in purines results in low urinary output, and pH less than 5.05 and all these factors leads to uric acid stones formation. Although most of uric acid stones are idiopathic but patients with gout may have uric acid stones. Uric acid stones are more prevalent in males pateints.[4]Autosomal recessive disorder cystinuria causes excess of cystine excretion in urine which is insoluble and leads to cystine stones which accounts for about 2% of all urinary system stones.[9,11]

Drug used for various ailments in patents, can also form stones in urinary system and which accounts about 1% of all urinary system stones. Common drugs which can cause these stones are Indinavir, atazanavir, sulfa drugs, triamterene and guaifenesin .[11]

Studies from India and abroad showed calcium, magnesium and oxalate were present in all the stones on chemical analysis.[12,13] A Nigerian study showed that urinary stones are more common in males with male to female ratio of 12:1. These stones were more frequently present in upper urinary tract (70.9 %) as compared to lower part of the tract (29.1 %). The frequency of different stones was calcium containing stones76.9 %, urate 16.3 %, struvite 4.3 %, xanthine 1.7 % and cystine 0.9 %. In a Canadian study, phosphate stones were heavier and relatively more common in females and in early age as compared to oxalate stones. While oxalate stones were light in weight and became more common with time. The diet consumed in particular areas also effect the composition of the urinary stones and in tropics due to less water consumption, increases the chances of stone formation.[14]

Studies from Durban area of South Africa showed high incidence of urinary system stones & calcium oxalate were more prevalent (80 %) followed by urate stones while incidence of phosphate (struvite) stones was low. The Saudi Arabian study reported that family history of hypercalciuria and hyperuricosuria had correlation with obesity and risk of urinary system stones in both sexes. From Japan reported frequency of calcium oxalate stones was 81.6% & urate stones was 15.8 %. In Berlin, renal stones were more frequent than Moscow and in Moscow struvite stones were due to infection by Proteus and E. coli bacteria. Studies from Pakistan reported that calcium oxalate calculi were the commonest variety.[15]

The diagnosis of nephrolithiasis is often made on the basis of clinical symptoms alone. However, various Urological associations recommends the following laboratory tests in all patients with an stone illness:[16]. Urinary sediment/dipstick test: To demonstrate blood cells, with a test for bacteriuria (nitrite) & urine culture in case of a positive reaction. Serum creatinine level: To measure renal function. Other laboratory tests that may be helpful includes CBC with differential in febrile patients. Serum electrolyte assessment in vomiting patients. Serum and urinary pH level may provide insight regarding patient's renal function and type of calculus.

Plain x-ray abdomen of KUB region is useful in the evaluation of urinary stone disease and in follow up of radiopaque stones. Abdominal ultrasonography now a days is used as initial investigation in the diagnosis and management of urinary system stones. Intravenous urography has been the standard imaging modality for urinary tract calculi. But non-contrast helical CT is being used frequently in the initial diagnosis & assessment of urinary system stones. Although NCCT is accurate and fast and visualizes the stones in whole of urinary system but it is costly and may not be available at all centres. The sensitivity of CT scan ranges from95 to 100 % with specificity between 94% to 96%.[17]

Advantage of CT scan is that it can show renal enlargement, hydro-ureteronephorosis/ pyonephrois, perinephric or periureteral inflammation. And Hounsfield units of stones can distinguish between different types of stones .[18]

MATERIAL & METHODS

This prospective study was conducted after the approval from ethical committee of the institution and written informed consent from the patients, who reported in single surgical unit of dept. of surgery, at Indira Gandhi Medical College Hospital, Shimla, between June 2017 to June 2018.

Renal stones from 50 patients were collected, & stones were chemically analyzed by Fourier transform infrared spectrometry. The data was correlated with data of the patients regarding age, sex, socio-economic class & factors like dietary habits, obesity, and with stone numbers.

Post operatively removed stone specimens were washed with sterile water to remove blood. Stones were first examined for shape, size and color. After noting the morphological features, a single stone from each patient (heaviest one from multiple stones) was chosen for chemical analysis. All stone samples were transferred into a sterile plastic container bearing the name, age and sex of the patient as well as the date and later

subjected to FTIR Spectroscopic analysis for the identification of various bio-chemical components. For FTIR analysis, all stone samples were sent for analysis to National Reference Laboratory, Dr Lal Path Labs at Rohini, New Delhi which is a NABL and ISO accredited and certified laboratory. In FTIR method, infrared radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some passes through the sample (transmitted). The resulting spectrum represents the molecular absorption and transmission pattern, creating a molecular fingerprint of the sample. Like fingerprints, no two unique molecular structures produce the same infrared spectrum. The pattern of infrared spectrum produced by the renal stones helped to identify the biochemical composition.

In this study, we used the Modified B.G. Prasad scale to define the socio- economic class of the patient [19]. Revised WHO BMI range for Asia- Pacific region was used in this study, for calculation and categorization of Body Mass Index of the patients.[20] We used 2014 Workers' Compensation Board of Nova Scotia levels, for assessment of physical activity level of the patients.[21]

Statistical Analysis

SPSS software version 8 was used to find the associations between patient age, sex/gender, socio-economic status, food habits with color, numbers and chemical composition of renal stones.

RESULTS

This was an observational study of 50 samples of renal stones removed from patients at Indira Gandhi Medical College Shimla, between the period from June 2017 to June 2018.

Renal stone samples were chemically analyzed by Fourier transform infrared spectrometry. The obtained data was correlated with regards to age, sex, socioeconomic class, dietary habits and obesity of patients and the following observations were made:

In our study, youngest patient was 4 years old and oldest was 84 years old. Maximum patients fell in the age group of 41-60 years (42 %). Mean age of the patients was 41.84years (SD ± 18.28).

Majority i.e. 66 % were males, while 34% were female. Among females 70.59 % had calcium oxalate stones, 17.65 % had urate stones and 5.88% had calcium oxalate phosphate containing stones. Among males 59.38 % had calcium oxalate stones, 15.63% had calcium magnesium phosphate stones, 6.25 % had urate stones, 12.50 % had calcium oxalate phosphate containing stones and 6.25%had calcium phosphate stones (Table 1).

In our study, 34% patients belonged to pre-obese and obese group with BMI >25Kg/m^2. Of the remaining patients, 48% had BMI in normal range and 18% had BMI in underweight category. Calcium oxalate stones were predominant in all categories of BMI under study (Fig. 1).

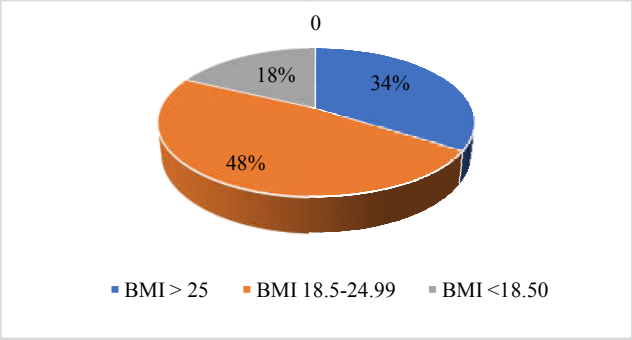

Figure 1 Showing Distribution of Patients according to BMI

In our study 48% patients were from lower middle class, while 18% of patients were from Middle class, 14% & 10% patients were from upper middle class & lower class respectively.

In our study, 66% of patients with renal stones were non-vegetarian while 34% were vegetarians. Among vegetarians 62.50% had calcium oxalate stones, 18.75% had calcium magnesium phosphate stones, none had urate stones, 6.25% had calcium oxalate phosphate containing stones and 6.25 % had calcium phosphate stones (Fig.2.). Among non-vegetarians 63.64 % had calcium oxalate stones, 6.66 % had calcium magnesium phosphate stones, 15.15 % had urate stones, 9.09 % had calcium oxalate phosphate containing stones and 6.02 % had calcium phosphate stones.

Table 1 Showing Gender Wise frequency of Different types of Renal Stones based on Chemical Analysis

Sex	No.	Calcium Oxalate	Calcium Magnesium Phosphate	Urate	Calcium Magnesium Phosphate Urate	Calcium Oxalate Phosphate	Calcium Phosphate	Calcium Oxalate and Urate	Total
Female	17	12	0	3	1	0	0	1	**17**
Male	33	19	5	2	0	4	2	1	**33**
Total	50	31	5	5	1	4	2	1	50

Figure 1. Showing Distribution of Patients according to BMI

Table 2 Showing distribution of renal Stones as per Socioeconomic Status (Modified B.G. Prasad scale) of the Patients

Socio- Economic Status	Frequency	Percent
LM (Lower Middle Class)	24	48
LO (Lower Class)	5	10
MM (Middle Class)	9	18
UM (Upper middle class)	7	14
U (Upper class)	5	10
Total	50	100.0

Figure 2. Showing Distribution of various Stone Types according to Diet

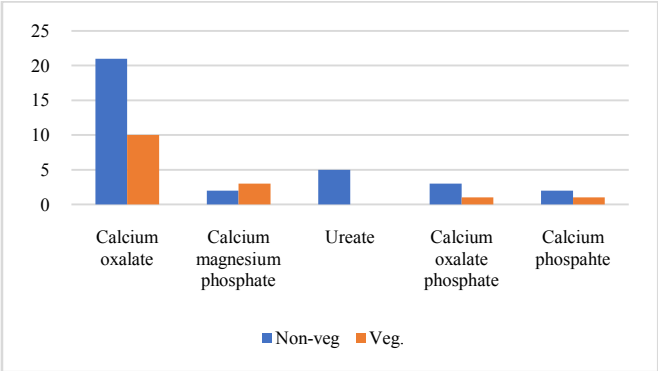

Figure 2 Showing Distribution of various Stone Types according to Diet

On chemical analysis, it was observed that 62 % of the patients had calcium oxalate stones, 10 % had calcium magnesium phosphate stones while urate and mixed stones (calcium magnesium phosphate, calcium oxalate phosphate, calcium phosphate urate and calcium oxalate and urate stones) were 10 % and 18% respectively .Among all the stones, calcium oxalate stones 39 %, calcium magnesium phosphate 80 %, calcium oxalate phosphate stones 25 %, urate stones 80 %, & calcium phosphate stones33.4% were multiple. While calcium magnesium phosphate urate stones & calcium oxalate and urate stones, 100 % stones were single (Fig. 3).

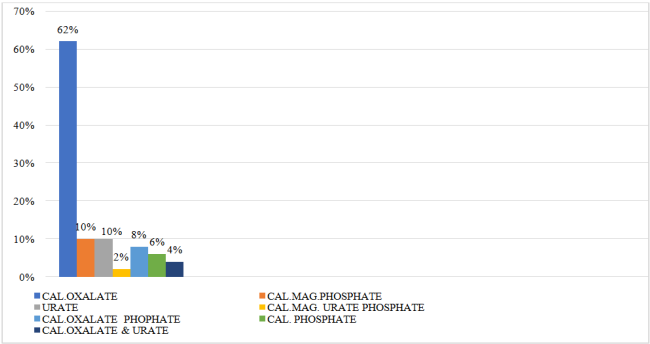

Figure 3 Showing Distribution of Renal Stones, based on their Chemical Analysis

DISCUSSION

In our study conducted on 50 patients, most of the patients were males (66 %), with male: female ratio of 1.94:1, most of the patients belonged to age group of 41 to 60 years (42 %) with mean age of 41.84 years. Other studies have also shown the same trend and reported more prevalence of renal stone disease in males as compared to females & life time prevalence was estimated at 13 % for men and 7 % for females. It is more common in age group of 30-60 yrs. It is more common in adult persons than in elderly. Renal stones are more frequent in persons living in hot, arid areas. [18]

Contrary to overall increased incidence of renal stones in males as shown by our study, the preponderance of infection stone was more in females (1:1.8) which is in conformity with other studies.[22] The increased incidence of infection stone in females, may be due to increased incidence of recurrent urinary tract infection in them which is due to close proximity of urethra to anus and sexual activity additionally serves to increase chance of bacterial contamination of female urethra. The pregnancy causes anatomical and hormonal changes that favor development of urinary tract infection. A change in genitourinary tract mucosa due to menopause may play a role in colonization of the introitus by coliforms, a major background factor for recurrent bladder infection in females.[23] In this study, 34 % patients were in pre-obese and obese groups with BMI > 25Kg / m^2. Of the remaining patients, 48 % had normal range BMI and 18 % were in underweight category. We found no correlation of increased BMI with increase in incidence of renal stones. However, a study concluded that obesity to be an independent risk factor for kidney stone formation.[21] In two large prospective cohort studies of men and women, the prevalence and incident risk of stone disease were directly correlated with weight and body mass index (BMI) in both sexes, and it was found that patients with higher BMI had more predisposition for kidney stones, although the magnitude of the association was greater in women than men.

In our study, most patients were from lower middle class (48 %), middle class patients were 18 % while14 %, 10 % each were from upper middle, lower upper classes respectively. Our results were in accordance with other studies,[24] that renal stones are less common in low socioeconomic class of population.

In our study, 66 % of patients with renal stones were non-vegetarian while 34% consumed vegetarian diet. This finding is in accordance with other studies that diets with increased animal proteins, low dietary magnesium, low dietary potassium, and increased sodium lead to renal stone formation[25] and same relation is advocated by the study conducted by Adair LS *et al*,[26] that kidney stones were more common in non-vegetarian group and moreover calcium stones were more common in both the groups. Among vegetarians 62.50 % had calcium oxalate stones, 18.75 % had calcium magnesium phosphate stones, none had urate stones, 6.25 % had calcium oxalate phosphate containing stones and 6.25 % had calcium phosphate stones. Among non-vegetarians 63.64 % had calcium oxalate stones, 6.66 % had calcium magnesium phosphate stones, 15.15 % had urate stones, 9.09 % had calcium oxalate phosphate containing stones and 6.02 % had calcium phosphate stones.

On chemical analysis of stones, it was found that, 62 % of the patients had calcium oxalate stones, 10% had calcium magnesium phosphate stones, 10 % had urate stones and 18% patients had mixed stones respectively. The study from Yemen, showed the same result of chemical analysis of stones i.e. 54.6 % calcium oxalate, 6.3 % uric acid, 0.7 % calcium phosphate, 29.6 % calcium oxalate and uric acid, 6.7% calcium oxalate and calcium phosphate, 1 % calcium oxalate and calcium carbonate, 0.5 % calcium oxalate, calcium phosphate and uric acid, 0.5 % calcium oxalate, calcium carbonate and uric acid, and 0.2 % calcium phosphate and uric acid. In conclusion, calcium oxalate was the most predominant chemical compositions in stones and it represents common urological problem.[27]

According study from Pakistan, most of the stones examined were of calcium oxalate, followed by mixed stones of calcium oxalate & calcium phosphate, pure uric acid and mixed lithiasis.[28] Similarly Coe F. *et al*., reported that calcium stones comprises about 80% of all urinary stones .[29]Also, in our study, among females 70.59 % had calcium oxalate stones, 17.65 % had urate, 5.88 % had calcium oxalate phosphate containing stones and among males 59.38 % had calcium oxalate stones, 15.63 % had calcium magnesium phosphate

stones, 6.25 % had urate stones, 12.50 % had calcium oxalate phosphate containing stones and 6.25 % had calcium phosphate stones respectively.

The frequencies of different stone types as reported in a large prospective cohort study performed in the United States, in men were calcium 71.5 %, uric acid 23.1 %, struvite 5 % and cystine 0.5 %. In women, the composition of stones was calcium 86.2 %, uric acid 11.3 %, struvite 1.3 % and cystine 1.3 %.[30]

In our study, 38 % of renal stones were multiple and 62% patients had single renal stone. Among calcium oxalate stones, 12 were multiple and 19 were single, among calcium magnesium phosphate stones, 80 % were multiple and 20 % were single, among urate stones, 80 % were multiple and 20 % were single, among calcium magnesium phosphate urate stones, 100 % were single, among calcium oxalate phosphate stones, 25 % were multiple and 75 % were single, among calcium phosphate stones, 66.6 % were single and 33.4 % were multiple and among calcium oxalate and urate stones, 100 % were single. This was in accordance with the result of other studies, that, tendency of single stone formation was higher i.e. 64.63 % as compared to double 22.05 % or multiple stones 13.30 % in all age groups of both sexes.[28]

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