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Original Research Article

# Assessment of Chemical Analysis of Kidney Stones in Tribal Population of Eastern Gujarat

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**Abstract**

**Introduction:** Kidney stone disease (KSD) is a third most common urinary tract disease worldwide. Its prevalence is exceeded only by urinary tract infections and pathologic conditions of the prostate. Dietary patterns, quality of drinking water and local varieties of vegetables, foods decide the chemical composition of kidney stones. This is responsible for the localized variations in the types of stones that are found in different geographical regions all over the world. Hence, we planned to study the types, morphological appearances, and chemical compositions of renal stones in patients operated in eastern Gujarat's populations which are mostly tribal.

**Materials and Methods:** Stones retrieved after open laparotomy were analyzed for their morphological appearances, chemical compositions, etc. in the department of Biochemistry of a tertiary care hospital. All the tests performed were of qualitative nature. Chemical analysis was done for the presence of calcium, carbonate, oxalate, magnesium, phosphate, uric acid, ammonium, and cystine.

**Results:** The prevalence of KSD was more in males (70.6%). Highest number of stones were found in the age group of 31 – 40 Years. Calcium oxalate was observed to be the most common type of stone. The chemical components were found in the decreasing order of frequency as follows – calcium (98%), oxalate (68%), magnesium (64%), phosphate (35%), carbonate (14%), cystine (4%), ammonium (nil).

**Discussion:** Risk factors for kidney stones are idiopathic hypercalciuria, followed by unduly acidic urine pH and hyperuricosuria. Lack of wide variety of vegetables in diet leads to consumption of oxalate rich foodstuffs and poor water intake ensures that the extra chemicals that are not flushed out of the system through urine and get collected in the kidneys. These collected chemicals then get converted into crystals and harden into stones.

**Conclusion:** Tribal belt where limited variety of vegetables and high TDS drinking water seem to be responsible for most kidney stones observed in these areas. Changes in the dietary habits like avoidance of oxalate-rich vegetables and adequate water intake of normal-TDS drinking water can be helpful to prevent the formation of kidney stones in population eastern part of Gujarat.

**Keywords:** Kidney stone disease, renal stones in tribals, kidney stone composition.

## Introduction:

Kidney stone disease (KSD) is a crystal concretion formed usually within the kidneys. Kidney stones are mainly lodged in the kidney and urinary bladder.[1] Mankind has been afflicted by urinary calculi since centuries dating back to 4000 B.C.[2] Kidney stone diseases are the third most common disease of the urinary tract system.[3] Although most patients with early KSD having with minor kidney injuries are treated effectively, it has been associated with an increased risk of end-stage renal failure.[4]

KSD is a multi-factorial disorder that is associated with biochemical, environmental, epidemiological as well as genetic risk factors for the pathogenesis.[5] The mechanism of stone formation is a complex process which results from several physicochemical events including supersaturation, nucleation, growth, aggregation, and retention of urinary stone constituents within tubular cells. The most common type of kidney stone is calcium oxalate formed at Randall's plaque on the renal papillary surfaces. These steps are modulated by an imbalance between factors that promote or inhibit urinary crystallization.

The prevalence of KSD is high in the developed as well as developing nations due to change in lifestyle. The incidences are also reported from across the geographical locations.[6] Kidney stones are found in patients with specific metabolic abnormalities, such as hypercalcemia, hyperuricosuria associated with calcium stones and with uric acid stones respectively due to primary hyperparathyroidism.[7]

The high incidence of urinary calculi in the eastern Gujarat tribal population, needs to be evaluated for possible etiopathogenesis. Apart from the routine etiologies, atypical dietary pattern can be a cause in this geographical terrain. Hence there is a need for the detailed characterization of the stone composition and to correlate it with habits of tribal people. Hence, we investigated the morphological and chemical characterization of kidney stones to get an insight into the etiology of the specific type of stone formation.

### Aim & Objectives:

- To analyze the chemical composition in kidney stones in tribal patients
- To analyze the morphological types and chemical composition of kidney stones.
- To predict possible etiologies of KSD specific for eastern Gujarat’s tribal population.
- To create awareness about kidney stones among tribal people.

### Materials and Methodology:

**Study Design:** single arm analytical study of chemical composition of renal stones.

**Study setting:** Department of Surgery provided the kidney stones removed from tribal patients and the department of Biochemistry performed the chemical analysis of stones at Zydus Medical College & Hospital Dahod, Gujarat, India. Period of the study was between January 2021 to June 2021.

### Inclusion and Exclusion Criteria:

The renal stones of tribal patients obtained by surgical intervention of open laparotomy were included in the study. Stones partially broken, damaged due to physical or chemical trauma were excluded. Stones removed by surgical procedures other than open laparotomy were excluded. Stones of the non-tribal patients were excluded from the study.

### Ethical Consideration:

The study was conducted after a due approval from the Institutional Ethics Committee of Zydus Medical College and Hospital as per the letter IEC No. 41/2020 dated 05/12/2020

**Sample Size:** Total number of stones included over the span of six months were 51.

**Preliminary examination of stones:**

The stones retrieved during open laparotomy surgeries were washed with distilled water to remove any blood or attached tissue immediately after removal. Then the stones were dried on a filter paper and sent to the Department of Biochemistry in a Sterile container. All the stones were observed for any physical as well as chemical damages in the laboratory. They were weighed. Morphological features of each stone (single or multiple, rough, smooth, mulberry, hard or waxy etc.) were noted. The stones were later pulverized and analyzed to ascertain their chemical composition. The qualitative analysis of chemical composition was based on the methods described by Hodgkinson[8].

**Heating on a platinum foil:**

A small quantity of the powder was heated to a dull red heat on a platinum foil. There is usually a little charring since most calculi contain some organic matter. The absence of a deposit after heating indicates the presence of organic components like uric acid, cystine, or, more rarely, xanthine, fibrin, sulphonamide, or fats. If a deposit remains after heating, this indicates the presence of inorganic salts. Cystine burns with a pale blue flame having a sharp smell and fibrin with a yellow flame having a smell of burnt feathers. Uric acid, ammonium urate, and xanthine burn without producing a flame.

**Carbonate and oxalate ions:**

A few grams of stone powder were placed over a platinum foil. A few drops of 2N HCI to a small portion of the powdered stone. An effervescence shows the presence of carbonate and this, in turn, suggests the presence of apatite since carbonate is invariably present as a carbonate-apatite in urinary calculi. If any ash remained after heating on a platinum foil, then it was transferred over on a glass side. A cover slip was kept over the ash. Few drops of 2N HCl were added to the residue. Appearance of bubbles were observed for. Any effervescence at this point when there was none before heating shows the presence of oxalate.

**Ammonium ion**

When powdered stone was slowly heated with 10% (w/v) potassium hydroxide solution, evolution of ammonia indicated the presence of magnesium ammonium phosphate (triple phosphate). Ammonia was easily detected by its smell. Alternatively, by holding a piece of damp red litmus paper at the mouth of the test tube ammonia was detected.

**Uric acid and Xanthine**

Small amount of the stone powder was put in a porcelain bowl. Two drops of concentrated nitric acid were added to it. The bowl was provided a mild heating by a small flame so that evaporation took place. The remaining red to yellow residue which changed to purplish red on cooling and by adding a drop of 2N ammonium hydroxide solution indicated the presence of uric acid. Whereas if xanthine was present, only yellow residue was observed that changed to orange on the addition of alkali and to red on warming.

**Cystine**

Few grams of stone powder were allowed to dissolve in 1 ml of 2N HCl. Then 1 ml of 2N NaOH was added to neutralize, followed by 1 ml of 5% (w/v) sodium cyanide solution. After allowing to stand for a few minutes, few drops of 5 % (w/v) sodium nitroprusside solution were added. A strong magenta colour indicated the presence of cystine.

**Calcium**

The stone powder was dissolved overnight. The homogenous solution was run as a sample on Erba EM200 Fully Automatic Chemistry analyzer. Calcium reagent of Erba XL containing Arsenazo III and phosphate buffer was used for qualitative analysis. Method's lower detection limit was 0.6 mg/dl. Any result more than 0.6 mg/dl was considered as positive for the presence of calcium in stone powder solution.

**Magnesium**

The homogenous liquid was run as a sample on Erba EM200 Fully Automatic Chemistry analyzer. Magnesium reagent of Erba XL containing xylidyl blue was used for qualitative analysis. Lower detection limit of the method was 0.16 mg/dl. Any result more than 0.16 mg/dl was considered as positive for the presence of magnesium in stone powder solution.

**Observations and Results:**

The Observations & Results of our study are as follows:

**Table 1: Age wise distribution of cases of kidney stone**

| Age | Frequency | Percent |
|---|---|---|
| 1-10 | 8 | 15.69 |
| 11-20 | 3 | 5.88 |
| 21-30 | 9 | 17.65 |
| 31-40 | 15 | 29.41 |
| 41-50 | 4 | 7.84 |
| 51-60 | 7 | 13.73 |
| 61-70 | 5 | 9.80 |
| Total | 51 | 100 |

**Table 2: Gender wise distribution of cases of kidney stone**

| Sex | Frequency | Percent |
|---|---|---|
| Female | 15 | 29.4 |
| Male | 36 | 70.6 |
| Total | 51 | 100 |

**Table 3: Weight wise distribution of kidney stones**

| Weight (gm) | Frequency | Percent |
|---|---|---|
| <10.00 | 40 | 78.43 |
| 10.00-19.99 | 8 | 15.69 |
| 20.00 & above | 3 | 5.88 |
| Total | 51 | 100 |

**Table 4: Frequency distribution of kidney stone's Colour appearance**

| Colour | Number | Percentage |
|---|---|---|
| Black | 3 | 5.9 |
| Grey | 17 | 33.3 |
| Brownish | 13 | 25.5 |
| Brownish grey | 4 | 7.8 |
| Greyish white | 4 | 7.8 |
| White | 10 | 19.6 |

**Table 5: Frequency distribution of kidney stone's morphological appearance**

| Colour | Number | Percentage |
|---|---|---|
| Smooth | 17 | 33.3 |
| Granular | 6 | 11.8 |
| Rough | 17 | 33.3 |
| Rough scaly | 7 | 13.7 |
| Zig zag | 4 | 7.8 |

**Table 6: Distribution of stone composition in male and females in the tribal population of eastern Gujarat.**

| | | Calcium | Carbonate | Oxalate | Magnesium | Phosphate | Uric Acid | Cystine | Ammonium |
|---|---|---|---|---|---|---|---|---|---|
| Female | No. | 15 | 2 | 8 | 11 | 5 | 6 | 0 | 0 |
| | **%** | **29.4** | **3.9** | **15.7** | **21.6** | **9.8** | **11.8** | **0** | **0** |
| Male | No. | 35 | 5 | 27 | 22 | 13 | 9 | 2 | 0 |
| | **%** | **68.6** | **9.8** | **52.9** | **43.1** | **25.5** | **17.6** | **3.9** | **0** |
| **Total** | No. | 50 | 7 | 35 | 33 | 18 | 15 | 2 | 0 |
| | **%** | **98.0** | **13.7** | **68.6** | **64.7** | **35.3** | **29.4** | **3.9** | **0** |

**Discussion:**

In our study, occurrence of kidney stones was observed to be more common among males than females. Similar prevalence was observed by Stapleton FB, et al; and Singh, et al.[9,10] Out of the 51 kidney stones analyzed, 36 (70.6%) were found in males and 15 (29.4%) were found in females. Similar prevalence was observed by Nidumuru S. et al (2016), where out of 43 stones analyzed, 39 were found in males (90.69%) and 4 were found in females (9.31%).[11]

In our study, highest number of stones were found in the age group 31 – 40 (29.41%) and least frequency of kidney stones were found in the age group 11-20 (5.88%). Similar prevalence was observed by Nidumuru S. et al (2016), where highest was observed in age group 35-40 years (40%) and least in less than 30 years (2%).

As observed in the present study, among all the kidney stones studied calcium composition accounted for almost 98% followed by oxalate stone (68%), magnesium ion (64.7%), phosphate (35.3%), uric acid (29.4%), carbonate ions (13.7%), cystine (trace amount 3.9%) and ammonium ion as nil. Similar composition was observed by Nidumuru S. et al (2016) where calcium oxalate (90%) and calcium phosphate (46%) were found.

Ansari et al, in their 1050 renal stone analysis from North Delhi showed calcium oxalate in 93.04% patients, uric acid stones in 0.95% and mixed pattern in 2.76% stones.[12] Similarly, Rao MVR et al., from Delhi has reported 96% of calcium oxalate stone in an analysis of 51 stones.[13] Urinary stone composition report showed predominance of calcium oxalate which was done by Ahlawat R et al, and Sharma RN et al., as 97% and 86.1% respectively.[14,15] Kumari A et al, in her studies has shown higher incidence of uric acid pure stones (18%) and mixed stones containing uric acid (52.3%).[16]

Out of 51 patients, 36 were males and 15 were females where increased incidence in males was attributed to increased dietary protein intake, which increases urinary excretion of phosphates and magnesium and reduces urinary citrate concentration. The lower risk of stone formation in women were initially attributed to increased urinary citrate concentrations due to the lower urinary saturation of stone forming salts. Endogenous estrogen and estrogen treatment in post-menopausal women may also decrease the risk of stone recurrence by lowering urinary calcium and calcium oxalate saturation.[17,18]

Low fluid intake greatly increases the risk of developing virtually all types of stones. For this reason, individuals at risk of developing stones are often advised to increase their fluid intake. High intakes of sodium and protein may also increase the risk of calcium oxalate stones. Oxalate-rich foods such as spinach and cocoa may also increase the risk of developing calcium oxalate stones.

## Conclusion:

In our observational study we found presence of calcium in almost 98% of stone with decreasing amount of oxalate, magnesium ion, phosphate ions, uric acid, carbonate ions, cystine and nil ammonium ion in the renal stone studied.

In male, kidney stone with Oxalate composition account for 77% while in female, stones with uric acid composition are highest with 40% prevalence. The major risk factors for

calcium oxalate stones are idiopathic hypercalciuria, followed by unduly acidic urine pH and hyperuricosuria. These kidney stone are formed by the extra chemicals that are not flushed out of the system through urine and get collected in the kidneys. These collected chemicals converted into crystals and harden into stones. Changes in the dietary habits and water intake can be helpful to prevent the formation of kidney stones. Thus, our study shows data based on composition and comparison of various kidney stones prevalent in the eastern Gujarat and helps to form scientific basis for further detailed research in this field.

## Limitations of Study:

The sample size of kidney stones was limited to around 50. For generalizing the results to the whole tribal population of eastern Gujarat, a large sample size of stones needs to be analyzed. The diet history, pattern of food consumption, water intake habits etc. was not available for correlation. Hence, these patterns / habits could not be associated with a particular type of stone. A study with larger sample size and detailed patient history shall be planned for the better correlation of composition of kidney stones with etiological factors associated in tribal population.

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