جامعة الملك سعود
King Saud University

IDENTIFICATION AND QUALITATIVE ANALYSIS OF RENAL CALCULI

BCH 472 [Practical]

Renal Calculi

- Kidney stones , renal calculi or renal lithiasis (stone formation) are small, hard deposits that form in the urinary system.
- The stones are made of **mineral** and **acid salts**.
- Kidney stones have many causes and can affect any part of your urinary tract (kidneys, ureters, bladder, and urethra).
- It is a common cause of **blood** in the urine and **pain** in the abdomen, flank, or groin.

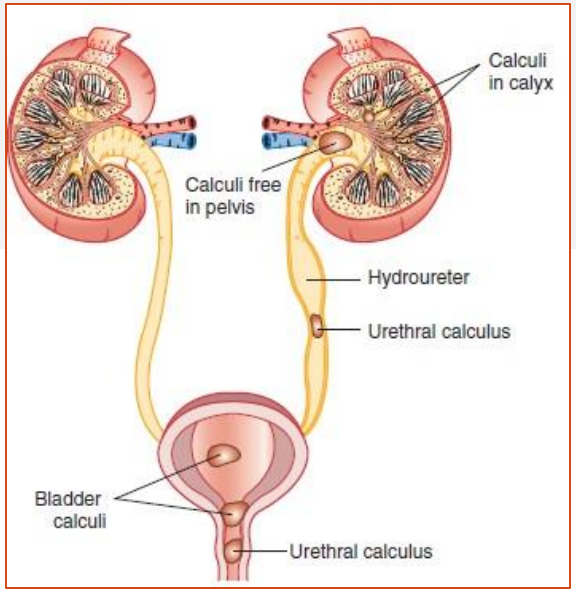


Figure 1. Location of calculi in the urinary tract

Pathogenesis of renal stones :

There are two basic aspects in the pathogenesis of renal stones:

1. Increased urinary excretion of stone forming elements: like calcium, phosphorus, uric acid, oxalate, and cysteine.

2. Low fluid intake: a low fluid intake results in the production of **concentrated urine**, causing super-saturation and crystallisation of stone-forming compounds. (*In addition, low urine flow rates favour crystal deposition on the urothelium*).

Note: Cystine stones formed only when its concentration increased in the urine.

3. Other: Physio-chemical changes which influence stone formation like: pH of urine, stone matrix, and protective substances in the urine.

Risk factors

1. Low fluid intake:

- The single **most important** determinant of stone formation is low fluid intake.
- A low fluid intake results in the production of concentrated urine.

Low fluid intake → Concentrated urine → Deposition of mineral and salts → Kidney stones

2. High salt diet.

3. Repeating, or recurrent, urinary tract infections.

4. Blockage of the urinary tract.

Investigation of Renal Calculi:

1- Urine analysis and Urine culture:

- It may show crystals, red blood cells, and/or pus cells in urine.

2- Stone analysis:

- Chemical analysis of stones (*simple test but is not an accurate*).
- Crystallography (*more accurate method*).

3- Biochemical investigations:

- Serum **calcium**, **phosphorus**, **uric acid**, and renal function tests (RFT).
- 24-hour urine for **calcium**, **phosphorus**, **uric acid**, **oxalate**, **citrate**, and **cystine**.
- Investigations for special clinical situations like **hyperparathyroidism**, **gout**, *should also be included*.

The main objectives in investigation are to find out :

1. The **composition** of stones.
2. **Cause** of stone formation.
3. **Functional status** of kidney.
4. Presence/absence of **obstruction** in urinary tract.
5. Evidence of possible **urinary infection**.

Types of Calculi

There are four basic types of kidney stones:

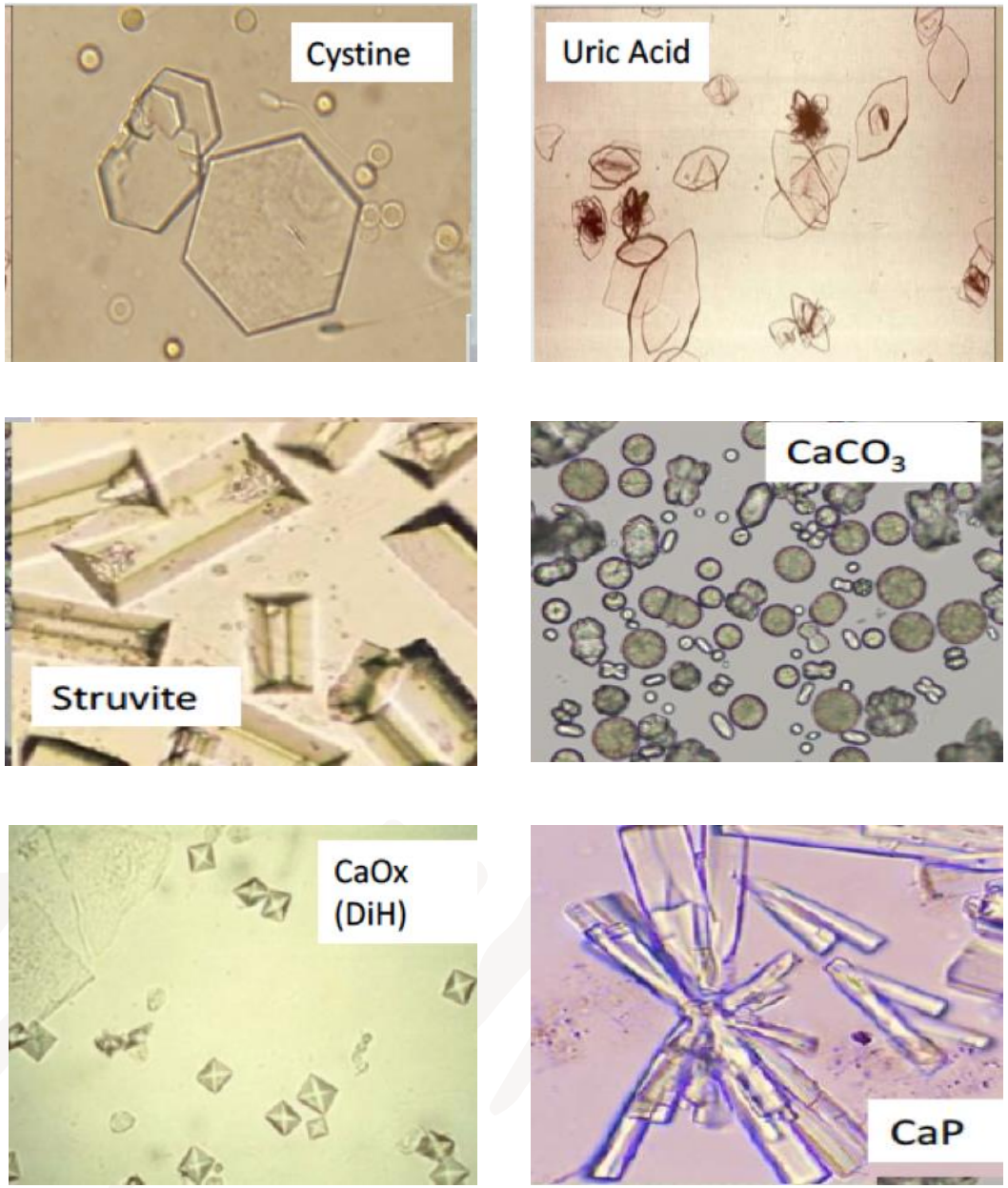
1. **Calcium** stones ➔ *calcium oxalate* and *calcium phosphate*.
2. **Uric acid** stones.
3. **Struvite** stones (*magnesium ammonium phosphate*), also known as **triple phosphate stone**.
4. **Cystine** stones.

- **Most kidney stones (70% to 80%) are calcium stones** – calcium oxalate, calcium phosphate, or a combination of the two materials.
- A patient can have one or a combination of these stones.

Types of Calculi

Stone type and composition	Contributing factors	Notes
Calcium stones **1. Calcium oxalate.** **2. Calcium phosphate.**	▪ Hyperparathyroidism. ▪ Hypercalcemia and Hypercalciuria. ▪ Hyperoxaluria (some food eg. spinach, beets and large doses of Vitamin C may increase the amount of oxalate in your urine). ▪ Vitamin D toxicity.	▪ **Calcium oxalate** stones are more common. ▪ **Calcium phosphate** stones are caused by the combination of high urine calcium and **alkaline urine** (*because phosphate level increase in alkaline urine*). ▪ **Carbonate apatite** is one kind of calcium phosphate stones, and commonly consider as infection marker.
Uric acid stones (Urate)	▪ Form in acid urine with pH around 5. ▪ Gout. ▪ High purine diet. ▪ Excessive urinary uric acid.	Can treated by: ○ Increase fluid intake. ○ Alkalinization of the urine.
Struvite stones (magnesium ammonium phosphate stones)	▪ Urea-splitting urinary tract infection UTIs (Some urinary bacteria can split the urea in urine to form ammonium and also to make urine less acidic).	▪ They can also be called **infection stones** if they occur with urinary tract infections (UTIs). Can treated by: ○ Increase fluid intake. ○ Acidification of the urine
Cystine stones	▪ Develop in patients with **cystinuria** (inherited disorder) **?**	▪ Less common. Can treated by: ○ Increase fluid intake. ○ Alkalinization of the urine.

Stones crystals shape



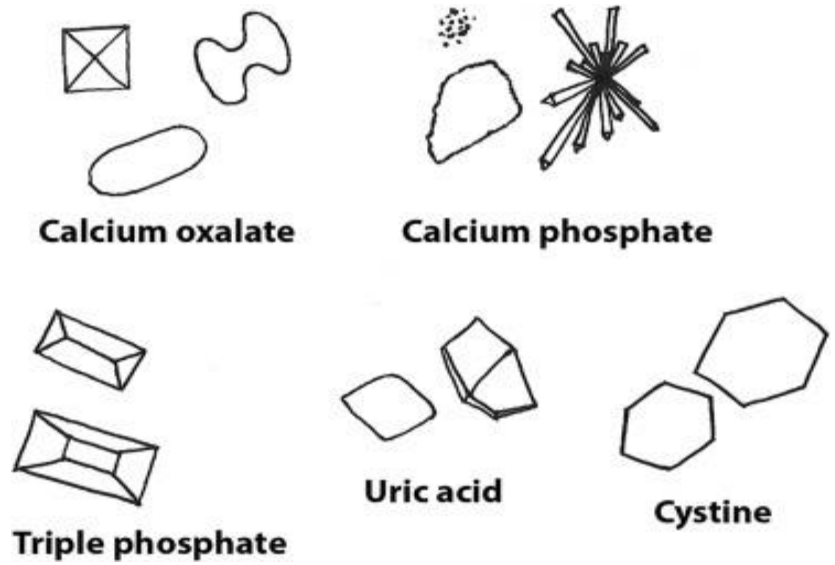


Figure 2. Different shapes of kidney stones

Practical Part

Objectives:

- Identification and qualitative analysis of renal calculi.

General Principle:

- Each test based on the chemical properties of the stone-forming substance.

Experiments:

1. Test for uric acid.
2. Test for carbonate.
3. Test for oxalate.
4. Test for phosphates.
5. Test for calcium.
6. Test for magnesium.

(1) Test for Uric acid:

Principle:

Uric acid undergoes oxidation when treated with HNO_3.

Method:

1. Take a small amount of the sample.
2. Add 5-7 drops of concentrated nitric acid (*Carefully*).
3. Heat in a water bath.

➔ (The positive result is yellow to orange color on the inner surface of the test tube)

(2) Test for carbonate:

Principle:

$2\ HCl + CaCO_3 \Longrightarrow CaCl_2 + H_2O + \mathbf{CO_2}$

Method:

1- Add 0.5 ml con. hydrochloric acid to a small portion of the sample (*Carefully*).

➔ (Gas bubbles will indicate the presence of carbonate).

(3) Test for oxalate:

Principle:

In sulfuric acid solution, oxalate combines with hydrogen to form oxalic acid.

$$2\,MnO_4^- + 5\,H_2C_2O_4 + 6\,H^+ \rightleftharpoons 2\,Mn^{2+} + 10\,CO_2 + 8\,H_2O$$

Purple color oxalic acid Colourless

Method:

1. Heat a part of the sample with 2 ml diluted sulphuric acid (2M H_2SO_4) for 1 min.

2. Add 2 drops (*one by one*) of potassium permanganate ($KMnO_4$) solution and mix.

➔ (The decolorization of potassium permanganate will confirm the presence of oxalate).

(4) Test for phosphates:

Principle:

Phosphate ions react with ammonium molybdate to produce a characteristic yellow precipitate of ammonium phosphomolybdate.

Method:

1- Dissolve a little of the sample in about 1.5 ml of concentrated nitric acid HNO_3.

2- Add an equal volume (1.5 ml) of ammonium molybdate solution.

3- Heat to boiling water bath.

➔ (If phosphates are present, a yellow precipitate of ammonium phosphomolybdate is obtained).

(5) Test for calcium:

Principle:

Calcium is precipitated as calcium oxalate using ammonium oxalate.

Method:

1- Dissolve small amount of the sample by heating with 2 ml dilute hydrochloric acid (2M HCL).

2- Add 1 ml ammonium oxalate.

➔ (A white precipitate of calcium oxalate shows the presence of calcium).

(6) Test for magnesium:

Principle:

The combination between titan yellow and **magnesium hydroxide** to produce an orange colour.

Method:

1- On a few amount of magnesium, add 1ml of titan followed by 1 ml potassium hydroxide *(to be strongly alkaline*).

➔ (An orange to red color indicates the presence of magnesium).

Results:

Component	Observation	Type pf stone/s
Uric acid		
Carbonate		
Oxalate		
Phosphate		
Calcium		
Magnesium		

Discussion:

- Comment on each result you obtained and mention whether the sample contains these component or not?
- What type of stone can be formed by each substance.
- Explain the possible causes of each type

References

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