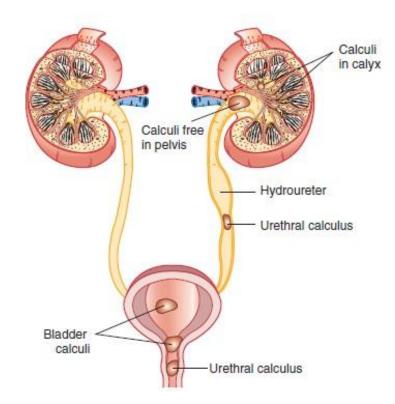
Identification and Qualitative Analysis of Renal Calculi

BCH472 [Practical]

-Renal Calculi:

- **Kidney stones** or **renal calculi** 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 <u>can affect any part of the urinary tract</u> (kidneys, ureters, bladder, and urethra).
- It is a common cause of **blood** in the urine and **pain** in the abdomen, flank, or groin.



Pathogenesis of renal stones :

- <u>There are two basic aspects in the pathogenesis of renal stones:</u>
- 1. Increased urinary excretion of stone forming elements: like calcium, phosphorus, uric acid, oxalate, and cysteine.

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



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.

- 2. <u>High salt diet.</u>
- 3. <u>Repeating</u>, or recurrent, urinary tract infections.
- 4. <u>Blockage of your urinary tract.</u>

-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:

1. Chemical analysis of stones (simple test but is not an accurate).

2. Crystallography (better method).

3- Biochemical investigations:

-Serum calcium, phosphorus, uric acid, and renal function tests.

-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 <u>four basic types of kidney stones</u> :
- 1. Calcium stones \rightarrow calcium oxalate and calcium phosphate.
- 2. Uric acid stones.
- 3. Struvite stones (magnesium ammonium phosphate).
- 4. Cystine stones.
- Most kidney stones (70% to 80%) are calcium stones calcium oxalate, calcium phosphate, or a combination of the two materials.

-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, strawberries 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 (calcium carbonate and calcium phosphate) is one kind of calcium phosphate stone.
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 kidney or urinary tract infections (UTIs). Can treated by: Increase fluid intake. Acidification of the urine
Cystine stones	• Develop in patients with cystinuria.	 Less common. Can treated by: Increase fluid intake. Alkalinization of the urine.

Practical Part

Objective:

• Identification and qualitative analysis of kidney stones.

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 HNO₃.

- 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.



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

(2) Test for carbonate :

- Principle:
- $2 \operatorname{HCl} + \operatorname{CaCO}_3 = = > \operatorname{CaCl}_2 + \operatorname{H}_2\operatorname{O} + \operatorname{CO}_2$
- Method:
- 1- Add 0.5 ml con. hydrochloric acid to a small portion of the sample (Carefully).
- \rightarrow (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.

 $2 \text{ MnO}_4^- + 5 \text{ H}_2\text{C}_2\text{O}_4 + 6 \text{ H}^+ \rightleftharpoons 2 \text{ Mn}^{2+} + 10 \text{ CO}_2 + 8 \text{ H}_2\text{O}$ Purple color oxalic acid colorless

• 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 (KMnO₄) solution and mix.

 \rightarrow (The decolonization 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 <u>ammonium phosphomolybdate</u>.

- 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.

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



(5) Test for calcium :

• Principle:

Calcium is precipitated as <u>calcium oxalate</u> 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.

 \rightarrow (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).

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





Component	Observation
Uric acid	
Carbonate	
Oxalate	
Phosphate	
Calcium	
Magnesium	

-Discussion:

- Comment in each results you obtained and mention whether the sample contains these component or not?
- What type of stone can be formed by each substance.
- What the disease that cause each type of stone.

Homework:

- A patient have been shown to has struvite stones (infection stone), what are your predictions for the following:
- 1. Urinalysis test.
- 2. Ultrasound imaging.

References:

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