Identification and Qualitative analysis of Renal Calculi

BCH 472
Renal Calculi:

- Kidney stones, renal calculi or renal lithiasis are small, hard deposits that form inside your kidneys.

- The stones are made of mineral and acid salts.

- Often, stones form when the urine becomes concentrated, allowing minerals to crystallize and stick together.

- A common cause of blood in the urine and pain in the abdomen, flank, or groin.

- Kidney stones have many causes and can affect any part of your urinary tract-kidneys, ureters, bladder, and urethra.
Pathogenesis of renal stones:

- There are **two basic aspects** in the pathogenesis of renal stones:
  
  - **Increased urinary excretion of stone forming elements** like calcium, phosphorus, uric acid, oxalate, and cystine
  
  - **Low fluid intake** (decreased urine volume). A low fluid intake results in the production of **concentrated urine**, causing **supersaturation** and **crystallisation** of stone-forming compounds.

- In addition, **low urine flow rates** favor crystal deposition on the urothelium.

- **Physio-chemical changes** which influence stone formation like: pH of urine, stone matrix, and protective substances in the urine.
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
• It is important to know the chemical composition of urinary stone to understand the cause and plan appropriate treatment.
• Chemical analysis of stones is a simple test but is not an accurate method. (will be done in today’s lab). Better method is crystallography.

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, renal tubular acidosis should also be included.
# Types of calculi

<table>
<thead>
<tr>
<th>Stone composition</th>
<th>Cause</th>
<th>Note</th>
</tr>
</thead>
</table>
| Calcium stone     | are the most common type of kidney stone and occur in two major forms: **calcium oxalate** and **calcium phosphate**. | *Hypercalciuria caused by:*  
Hyperparathyrodisim. Vitamin D toxicity.  
*Excess vitamin C is converted into oxalate and excreted in the gut and urine. And some food eg.spinach.* |
|                   | • **Calcium oxalate** stones are more common.  
• Calcium oxalate stone formation may be caused by high calcium “Hypercalciuria” and high oxalate Excretion.  
• **Calcium phosphate** stones are caused by the combination of high urine calcium and **alkaline** urine. | |
| Uric acid stones  | • form when the urine is persistently **acidic**.  
• If uric acid becomes **concentrated** in the urine, it can settle and form a stone. | *Excessive urinary uric acid: a diet rich in purines found in protein such as meats may increase uric acid in urine, Gout.* |
# Types of calculi

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<thead>
<tr>
<th>Stone composition</th>
<th>Cause</th>
<th>Note</th>
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</thead>
<tbody>
<tr>
<td>Carbonate apatite (calcium carbonate and calcium phosphate)</td>
<td>These stones develop as a consequence of recurrent or chronic urinary tract infections caused by urease producing bacteria.</td>
<td>Some urinary bacteria can split the urea in urine to form ammonium and also to make urine less acidic.</td>
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<td>Struvite (magnesium ammonium phosphate)</td>
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<tr>
<td>Cystine stone</td>
<td>develop in patients with cystinuria.</td>
<td>caused by mutations in the genes, encode for two parts of a transporter protein that is made primarily in the kidneys. These defects prevent proper reabsorption of amino acids.</td>
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<tr>
<td>TYPE OF STONE</td>
<td>CONTRIBUTING FACTORS</td>
<td>TREATMENT</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Calcium (oxalate and phosphate)</td>
<td>Hypercalcemia and hypercalciuria</td>
<td>Treatment of underlying conditions</td>
</tr>
<tr>
<td></td>
<td>Immobilization</td>
<td>Increased fluid intake</td>
</tr>
<tr>
<td></td>
<td>Hyperparathyroidism</td>
<td>Thiazide diuretics</td>
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<tr>
<td></td>
<td>Vitamin D intoxication</td>
<td></td>
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<td></td>
<td>Diffuse bone disease</td>
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<tr>
<td></td>
<td>Milk-alkali syndrome</td>
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<td></td>
<td>Renal tubular acidosis</td>
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<tr>
<td></td>
<td>Hyperoxaluria</td>
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<td></td>
<td>Intestinal bypass surgery</td>
<td></td>
</tr>
<tr>
<td>Magnesium ammonium phosphate (struvite)</td>
<td>Urea-splitting urinary tract infections</td>
<td>Treatment of urinary tract infection</td>
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<tr>
<td></td>
<td></td>
<td>Acidification of the urine</td>
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<tr>
<td></td>
<td></td>
<td>Increased fluid intake</td>
</tr>
<tr>
<td>Uric acid (urate)</td>
<td>Formed in acid urine with pH of approximately 5.5</td>
<td>Increased fluid intake</td>
</tr>
<tr>
<td></td>
<td>Gout</td>
<td>Allopurinol for hyperuricosuria</td>
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<tr>
<td></td>
<td>High-purine diet</td>
<td>Alkalization of urine</td>
</tr>
<tr>
<td>Cystine</td>
<td>Cystinuria (inherited disorder of amino acid-metabolism)</td>
<td>Increased fluid intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkalization of urine</td>
</tr>
</tbody>
</table>
Treatment

Includes relief of pain, **hydration**, dietary changes and Alkalization or acidification of urine (depend on the type of stone). The majority of stones pass spontaneously within 48 hours. However, some stones may not. If a stone does not pass, **urologic intervention** may be needed.

**Management**

General risk factors leading to **calculi** development are stasis of urine, high serum calcium or uric acid levels, vegetarian diet (changes urinary **pH**), high protein diet, UTI, abnormal urinary **pH** (urinary **pH** is normally around 5.85), deficiency of crystal-inhibiting factors, and low urine output. A urinary **pH** below 5.5 is a risk factor for uric acid stone formation, whereas a urinary **pH** above 7.5 is a risk factor for struvite stone formation. **Dietary changes** may be used to prevent the concentration of stone-forming crystals in the urine. A person with stones composed of calcium oxalate, for example, is encouraged to limit the intake of high oxalate foods such as spinach and chocolate. A person who has recurrent stone formation is encouraged to adopt a low-sodium low-protein diet. A high sodium intake increases the amounts of sodium and calcium excretion in the urine, increases the saturation of
Practical Part

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
Objective:

- Identification and Qualitative analysis of Renal Calculi, to find out the presence and composition of stones.
1) Test for Uric acid

**Principle:** Uric acid undergoes oxidation when treated with HNO$_3$.

**Method:**
1. Put a small amount of the sample.
2. Add 5-7 drops of concentrated nitric acid.
3. Heat in a water bath.

*yellow to orange color* on the inner surface of the test tube.
2) Test for carbonate

**Principle:**
2 HCl + CaCO3 --> CaCl2 + H2O + CO2

**Method:**
1-Add 0.5 ml of conc. hydrochloric acid to small portion of sample.

*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.

Potassium permanganate reacts with oxalate ions to produce carbon dioxide and water in an acidic solution, and the permanganate ion is reduced to manganese (II) as follows:

\[ 5C_2O_4^{2-} + 2MnO_4^- + 16H^+ \rightarrow 10CO_2 + 8H_2O + 2Mn^{2+} \]

The permanganate ion is intensely **purple**, whereas the manganese (II) ion is nearly **colorless**.

**Method:**
1-Heat a part of sample3 with 2 ml dilutes sulphuric acid (2M H2SO4) for 1 min.
2-Add 2 drops (one by one) of, potassium permanganate (KMnO4) solution and Mix

The **decolonization** and **evolution of bubbles** will confirm the presence of oxalate.
4) Test for phosphates

Principle:
Phosphate ions react with ammonium molybdate to produce a characteristic yellow precipitate, ammonium phosphomolybdate.

Method:
1-Dissolve a little of the sample in about 1.5 ml of concentrated nitric acid HNO₃.
2-Add an equal volume (1.5 ml) of ammonium molybdate solution.
3-Heat to boiling.

(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 5 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:
When magnesium hydroxide precipitated in the presence of titan yellow by sodium hydroxide the yellow color of reagent changes to red or orange-red.

\[ \text{Mg}^{2+} + 2\text{OH}^- \rightarrow \text{Mg(OH)}_2 \], titan yellow form a red absorption complex when magnesium hydroxide is precipitated in its presence.

Method:
1- add 1 ml of titan to small amount of sample 6.
2- add 1 ml of sodium hydroxide until strongly alkaline. A red or orange-red color indicates the presence of magnesium.
Results:

<table>
<thead>
<tr>
<th>Components</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td></td>
</tr>
<tr>
<td>carbonate</td>
<td></td>
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<tr>
<td>oxalate</td>
<td></td>
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<tr>
<td>phosphates</td>
<td></td>
</tr>
<tr>
<td>calcium</td>
<td></td>
</tr>
<tr>
<td>magnesium</td>
<td></td>
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</tbody>
</table>
Discussion:

Comment in each results you obtained and mention whether the sample contains these component or not? And the disease that cause each type of stone.
Questions:

How change in urine pH can influence the type of stone formed?

Why Hyperparathyroidism increases chance of calcium stone formation?