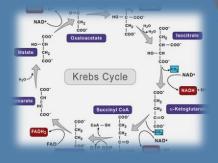## Lecture (1)

Estimation of Blood Calcium

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Calcium

Calcium is the most common mineral ion in the body and one of the most important. The body calcium is distributed as:

❶ **99%:** found in the bone and teeth it give the strength to the bone.

❷ **1%:** found in plasma.

The plasma calcium exists in three forms:

➤ <u>50 %</u> free or ionized calcium: it is physiologically active calcium whose concentration is finely regulated.

➤ <u>40 %</u> protein bound calcium: it is mainly bound to albumin and less to globulin.

➤ <u>10 %</u> complex "calcium-calcium": calcium bound to citrate, oxalate, bicarbonate, …., etc.

A test for calcium in the blood checks the calcium level in the body that is not stored in the bones. (the 1% that is present in the blood) because this part is important for normal:

1.heart function.

2.neurve function

3.muscular function .

Almost all of the calcium in the body is stored in bone. The rest is found in the blood.

Normally the level of calcium in the blood is carefully controlled. When blood calcium levels is low the bones release calcium to bring it back to a good blood level. When blood calcium levels is high the extra calcium is stored in the bones or passed out of the body in urine and stool. The *amount of calcium in the body depends* on the amount of:

- Calcium you get in your food.

- Calcium and vitamin D your intestines absorb.

- hormones, as parathyroid hormone,

- kidney function because the calcium is excreted from the kidney.

Normal serum calcium is 8.5-10.5 mg/dl

Blood calcium levels do not directly tell how much calcium is in the bones, but how much calcium is circulating in the blood.

When an abnormal total calcium result is obtained, it is viewed as an indicator of an problem. To help diagnose the problem, additional tests are often done to measure

- ➤ Ionized calcium,

- ➤ Urine calcium,

- ➤ Vitamin D, and

- ➤ Parathyroid hormone (PTH).

PTH and vitamin D are responsible for maintaining calcium concentrations in the blood normal.

Measuring urine calcium can help determine whether the kidneys are excreting the proper amount of calcium and testing for vitamin D, phosphorus can help determine whether other deficiencies or excesses exist.

Blood and urine calcium measurements cannot tell how much calcium is in the bones.

➤ **Causes and investigation of abnormal results**

A. Low values :

1. Causes

In the majority of instances there is clinical evidence of the cause of hypocalcaemia. More frequent causes include :

- Vitamin D deficiency (privational and functional).

- Renal failure.

- Pancreatitis.

- Hypomagnesaemia

- hypoparathyroidism.

2. Investigation:

Mild hypocalcaemia (>2.1 mmol/L), particularly in an asymptomatic individual, should be confirmed by a repeat test to exclude the result's being a statistical outlier. If the cause of hypocalcaemia is not obvious from clinical or other evidence, it should be investigated initially by measuring [creatinine], [magnesium], [PTH] and [vitamin D].

Except in hypoparathyroidism and hypomagnesaemia, [PTH] is usually increased in hypocalcaemia (secondary hyperparathyroidism).

B. High values :

1. Causes:

There are numerous causes of hypercalcaemia. The most frequent are:
- hyperparathyroidism (the commonest cause in asymptomatic individuals); this may be a component of multiple endocrine neoplasia (MEN), particularly in younger patients.
- malignancy (with or without osseous metastases; such patients often have clinical evidence of the underlying disease, of which squamous cell carcinoma is the most frequent).

Other causes include:
- Idiopathic hypocalciuric hypercalcaemia.
- Treatment with thiazide diuretics.
- Treatment with lithium.
- Sarcoidosis.
- Thyrotoxicosis.
- Adrenal failure.
- Use of 1α-hydroxylated derivatives of vitamin D e.g. in chronic kidney disease.

2. Investigation:

Mild hypercalcaemia (>2.70 mmol/L), particularly in an asymptomatic individual should be confirmed by a repeat measurement to exclude the result being a statistical outlier. Confirmed hypercalcaemia should be followed up by measurement of [PTH]: if undetectable, this suggests a cause other than hyperparathyroidism; if detectable, even if the result is within the reference range, hyperparathyroidism is the most likely diagnosis but familial hypocalciuric hypercalcaemia should be considered. In this condition, urinary [calcium]:[creatinine] ratio is low and other members of the family may have hypercalcaemia. In severe, symptomatic hypercalcaemia (e.g. with polyuria, polydipsia and dehydration, immediate assessment of renal function is required.

Estimation of blood calcium

➢ **Principle method:**

Calcium ions Ca^{2+} reacting with *O-cresolphthalein* complex in an alkaline solution to form an intense violet colored complex which is maximally absorbs at (578 n.m.).

➢ **Normal Value:**

- Serum: 8.5-10.5 mg/dl.

- Urine: 100-400 mg/24hrs.

➢ **Procedure:**

1. Mix reagent 1 (R_1) and reagent 2 (R_2) in the ratio 1:1.

2.

	Standard	Sample	Blank
	1000 µL	1000 µL	1000 µL
Standard	10 µL		
Serum		10 µL	

3. Mix and incubate for (5 min) at room temperature . then read the absorbance of the sample and standard against blank at wave length (578 n.m.).

4. Calcium standard concentration = 10 mg/dl.

5. Calculating the concentration of sample by the following equation:

$$\textbf{Calcium Concentration} = \frac{\textbf{Absorbance of Sample}}{\textbf{Absorbance of Standard}} \times \textbf{10}$$