Clinical chemistry

Calcium metabolism

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Calcium metabolism

- **Total body calcium:**

- Depends upon the calcium absorbed from dietary intake and that lost from the body.
- 98% of body calcium is found in skeleton.
- Although the extraosseous fraction of calcium account for 1% of total calcium, it is essential for neuromascular excitability and cardiac muscle.

❑ Factors affecting calcium intake

- 6-12 mmol (0.25 – 0.5 g) of the ingested calcium 25 mmol (1g) per day is absorbed.
- 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) (calcitriol), active metabolite of vitamin D, is needed for calcium absorption.

❑ Factors affecting calcium loss

Calcium is lost in urine and faeces

➢ Urinary calcium excretion depends on:

✓ The amount of calcium reaching the glomeruli

✓ Glomerular filtration rate (GFR)

✓ Renal tubular function

- Parathyroid hormone and 1,25-dihydroxyvitamin D increase urinary calcium reabsorption.

- Faecal calcium derived from diet & a portion of the large amount of intestinal secretions that has not been reabsorbed.
- Calcium in the intestine may form insoluble, poorly absorbed complexes with oxalate, phosphate or fatty acids.
- An excess of fatty acids in intestinal lumen in steatorrhoea may contribute to calcium malabsorption.

Concept of plasma calcium and albumin correction (adjusted)

- The normal plasma calcium concentration is tightly controlled within 2.15 – 2.55 mmol/l.
- Calcium in plasma found in two main forms:

✓ Calcium bound to proteins (predominantly albumin). Physiologically, it is inactive form and account for < 50% of total calcium concetration.

✓ Free ionized form (Ca^{2+}). Physiologically, it is active form and account for > 50% of the total calcium concentration.

Concept of plasma calcium and albumin correction (adjusted)

- Change in plasma total calcium (but not free ionized fraction) occur in parallel with change in plasma protein (particularly albumin) concentration.
- The plasma total calcium concentration is lower in the supine than in the erect position because of the effect of posture on fluid distribution and therefore on plasma protein concentration.
- For technical reasons, direct measurement of free calcium ionized fraction is confined to special cases e.g. acid-base disturbance.

Concept of plasma calcium and albumin correction (adjusted)

- In an attempt to calculate the active fraction of the plasma total calcium concentration, we commonly use the following formula:

Plasma albumin-adjusted or corrected calcium (mmol/l)=plasma measured calcium+(40-plasma albumin concentration)(g/l)x0.02

But this is not always reliable because binding is not always simple, particularly if extremes of plasma albumin concentration occur.

Concept of plasma calcium and albumin correction (adjusted)

- Changes in plasma hydrogen ion concentration affect the binding of calcium to plasma proteins because hydrogen ion compete with calcium ion for binding sites. The plasma total calcium concentration is unaltered by changes in hydrogen concentration.

Concept of plasma calcium and albumin correction (adjusted)

- In alkalosis, increases binding and so deceases the proportion of the free ionized form, despite a normal plasma total calcium concentration.
- An acidosis decreases binding and so increases the proportion of the free ionized form.

Control of plasma calcium

- In calcium homeostasis, extracellular calcium concentrations are controlled rather than the total body content. The effectiveness of this control depends on:

- An adequate supply of: calcium and vitamin D
- Normal function of the: intestine, parathyroid glands and kidney

An impairment in any of these factors, calcium leaves bone by passive physicochemical diffusion, and plasma concentration may be maintained at the expense of bone calcification.

1- Parathyroid hormone (PTH)

- It is a single-chain polypeptide containing 84 residues, with its 34 N-terminal amino acids largely determining its biological activity.
- It is metabolized by renal, hepatic and bone cells. Renal clearance from plasma of the physiologically inert C-terminal fragment is slower than that of the N-terminal fragment, which may accumulate in plasma in renal glomerular dysfunction.

1- Parathyroid hormone (PTH)

❑ The biological actions of PTH include:

- Stimulation of osteoclastic bone resorption, releasing both free ionized calcium and phosphate into the ECF ⟹ increases the plasma concentrations of both calcium and phosphate.
- Decreased renal tubular reabsorption of phosphate, causes phosphaturia and increased reabsorption of calcium ⟹ increase the plasma calcium concentration but decrease the phosphate.

1- Parathyroid hormone (PTH)

- Control of PTH secretion depends on the concentration of free ionized calcium in blood circulating through the parathyroid gland.
- Under physiological condition, increasing the rate of PTH secretion as a result of a fall in free ionized calcium concentration will continue until the calcium concentration return to normal.
- Extracellular magnesium concentration affect the secretion of PTH; PTH secretion decrease by sever, chronic hypomagnesaemia.

1- Parathyroid hormone (PTH)

- Detectable plasma PTH, even if the concentration is within the reference range, is inappropriate in the presence of hypercalcaemia and is consistent with primary or, more rarely, tertiary hyperparathyroidism.

2- Parathyroid hormone-related protein (PTHRP)

PTHRP is a peptide hormone that has a similar amino acid sequence at the biologically active end of the peptide, therefore activating the same receptors as PTH.

The gene that codes for PTHRP is widely distributed in body tissues but is normally repressed. However, it may become derepressed in certain tumours, causing humoral hypercalcaemia of malignancy.

3- Calcitonin

Calcitonin (produced in the C cells of the thyroid gland) decreases osteoclastic activity, slows calcium release from bone and has the opposite effect on plasma concentrations of PTH. It is probably less important than PTH in physiological homeostasis.

Exogenous calcitonin has been used to treat hypercalcaemia and Paget's disease of bone.

4- Metabolism and action of vitamin D

❑ Vit. D is derived from:

- ergocalciferol (vitamin D_2), obtained from plants in the diet.
- cholecalciferol (vitamin D_3), formed in the skin by the action of ultraviolet light on 7-dehydrocholesterol; this is the form found in animal tissues, especially the liver.

4- Metabolism and action of vitamin D

- Vit. D is transported in plasma bound to specific carrier proteins. It is inactive until metabolized.
- In the liver, cholecalciferol is hydroxylated to 25-hydroxycholecalciferol (25-OHD_3) by the enzyme 25-hydroxylase. The rate of formation of 25-OHD_3 is affected by the supply of substrate in the form of calciferol. It is the main circulating form and store of the vitamin. Other hydroxylated metabolites are found, such as 24,25-$(OH)_2D_3$.

4- Metabolism and action of vitamin D

- In the proximal renal tubular cells of the kidney, 25-OHD_3 undergoes a second hydroxylation, catalysed by the enzyme 1- α -hydroxylase to form the active metabolite 1,25-$(OH)_2D_3$.
- The activity of 1- α -hydroxylase, and hence the production of 1,25-$(OH)_2D_3$, may be stimulated by:

- A low plasma phosphate concentration.
- An increase in plasma PTH concentration, possibly because of its phosphate-lowering effect.

4- Metabolism and action of vitamin D

- ❑ The activity 1- α -hydroxylase is inhibited by:
  - Hyperphosphataemia.
  - High levels of free ionized calcium.
- ❑ The kidney is an endocrine organ, synthesizing and releasing the hormone 1,25-$(OH)_2D_3$; impairment of the final hydroxylation helps explain the hypocalcaemia of renal disease.
- ✓ This hormone increases calcium absorption by intestinal mucosal cells. In conjunction with PTH, it stimulates osteoclastic activity, releasing calcium from bone.

4- Metabolism and action of vitamin D

- ❑ The action of PTH on bone is impaired in the absence of 1,25-$(OH)_2D_3$.
- ✓ A fall in plasma free ionized Ca stimulates PTH secretion. The PTH enhances 1- α -hydroxylase activity and therefore stimulates 1,25-$(OH)_2D_3$ synthesis. The two hormones act synergistically on the osteoclasts of bone, releasing calcium into the circulation.

4- Metabolism and action of vitamin D

- In the short term, the homeostatic mechanisms involving the effects on bone are the more important.
- If hypocalcaemia is prolonged, more efficient absorption becomes important. Once the plasma free ionized calcium concentration is adjusted, the secretion of both PTH and 1,25-$(OH)_2D_3$ is suppressed.

4- Metabolism and action of vitamin D

- Thus, 25-OHD_3 is the circulating, inactive form of vitamin D and plasma concentrations fall in deficiency states. The measurement of the biologically active metabolite, 1,25-$(OH)_2D_3$, which circulates in plasma bound to vitamin D-binding protein (VDBP) in very low concentrations, is rarely indicated unless a defect in the vitamin metabolic pathway is suspected, as it does not reflect body stores.

4- Metabolism and action of vitamin D

- The vitamin D receptor (VDR) is found in almost all cell nuclei with various effector systems such as endocrine, paracrine or autocrine. Calcitriol activates this receptor.

5- Calcium-sensing receptor

- The calcium-sensing receptor (CaSR) is a G protein-coupled receptor. This allows the parathyroid cells and the ascending loop of Henle epithelial cells to respond to changes in extracellular calcium. The parathyroid cell surface is rich in CaSR, which allows PTH secretion to be adjusted rapidly depending on the calcium concentration.

5- Calcium-sensing receptor

- Defects in the CaSR gene are responsible for various rare defects of calcium homeostasis.

✓ Inactivating mutations include familial benign hypocalciuric hypercalcaemia and neonatal severe hyperparathyroidism.

✓ activating mutations include autosomal dominant hypocalcaemia with hypercalciuria.

- Calcimimetic agents have been devised that bind and activate the CaSR, resulting in decreased PTH release and reduced plasma calcium concentrations.

6- Miscellaneous mechanisms of calcium control

- Thyroid hormone excess may be associated with the histological appearance of osteoporosis and with increased faecal and urinary excretion of calcium, probably following its release from bone.
- Other hormones influencing calcium metabolism include oestrogens, prolactin and growth hormone. These may increase 1,25-$(OH)_2D_3$ production and increase calcium absorption during pregnancy, lactation and growth.

DISORDERS OF CALCIUM METABOLISM

- A low plasma free ionized calcium concentration normally stimulates PTH secretion, which results in phosphaturia; the loss of urinary phosphate over-rides the tendency to hyperphosphataemia due to the action of PTH on bone. Consequently :
  - ✓ The plasma phosphate concentration is usually low when the plasma PTH concentration is increased. Conversely, a high plasma free ionized calcium concentration, unless due to inappropriate excess of PTH, inhibits PTH secretion and causes a high plasma phosphate concentration.

DISORDERS OF CALCIUM METABOLISM

- Plasma calcium and phosphate concentrations usually vary in the same direction unless:

- renal glomerular dysfunction is severe enough to impair the phosphaturic (and therefore hypophosphataemic) effect of PTH or PTHRP,
- there is inappropriate excess or deficiency of PTH due to a primary disorder of the parathyroid gland or to secretion of PTHRP; in such cases calcium and phosphate vary in opposite directions.

Hypercalcaemia

- **Clinical effects of an increased plasma albumin adjusted calcium concentration**

1) Effect on renal

- *Renal damage* is one of the most serious clinical consequences of prolonged hypercalcaemia. (⇧ plasma free ionized calcium concentration, calcium phosphate precipitate in the kidney.

Hypercalcaemia

- *Polyuria*, characteristic of chronic hypercalcaemia, may result from impairment of renal concentrating ability owing to calcification of the tubular cells; acute hypercalcaemia may cause reversible inhibition of the tubular response to antidiuretic hormone rather than to cell damage. These effects can lead to dehydration.

Hypercalcaemia

- *Renal calculi*, without significant parenchymal damage. ⇧ Conc. of free ionized calcium in the glomerular filtrate ⟹ precipitate of calcium salt in the urine.
- *Hypokalaemia*, often with a metabolic alkalosis, is associated with hypercalcaemia. Calcium may directly inhibit potassium reabsorption from the tubular lumen.

Hypercalcaemia

2) Effect on neuromuscular excitability: High extracellular free ionized calcium concentrations can depress neuromuscular excitability in both voluntary and involuntary muscle.
3) Effect on central nervous system: Depression, anorexia, nausea and vomiting are probably caused by an effect on the CNS.

4) Effect on stomach: Calcium stimulates gastrin (and therefore gastric acid) secretion. There is an association between chronic hypercalcaemia and peptic ulceration.

Hypercalcaemia

5) Effect on blood pressure: Some patients with hypercalcaemia may be hypertensive.

6) Effect on the heart: Severe hypercalcaemia causes characteristic changes in the electrocardiogram (ECG). If plasma level exceed about 3.5 mmol/L, there is a risk of sudden cardiac arrest or ventricular arrhythmias.

7) Hypercalcaemia is also associated with bone and joint pain.

Causes of hypercalcaemia

- Overall, thiazides are one of the most common causes of mild hypercalcaemia. However, most causes of severe hypercalcaemia are related to either primary hyperparathyroidism or malignancy (80% are due to bony metastases, and 20% mainly due to ectopic PTHRP).
- True free ionized or albumin-adjusted hypercalcaemia with hypophosphataemia is usually caused by inappropriate secretion of PTH or PTHRP.

Causes of hypercalcaemia

- Inappropriate PTH secretion occurs in the following clinical situations:
  - production of PTH by the parathyroid glands due to:
    - primary hyperparathyroidism.
    - tertiary hyperparathyroidism.

Causes of hypercalcaemia

- If renal glomerular function is adequate, the high circulating PTH or PTHRP concentrations cause hypercalcaemia, which is associated with a low-normal or low plasma phosphate concentration in relation to GFR, and to phosphaturia. If glomerular damage develops due to hypercalcaemia, the kidneys cannot respond normally to the phosphaturic effect of PTH and, because of impaired hydroxylation of 25-OHD_3, plasma calcium concentrations may fall towards or within the reference range as renal failure progresses. Because plasma phosphate concentrations tend to rise, diagnosis may be difficult at this stage.

Causes of hypercalcaemia

❖ The clinical features of PTH- or PTHRP-induced hypercalcaemia are due to:

- Excess circulating concentration of free ionized Ca

  - As a consequence of increased osteoclastic activity and release of calcium from bone, and enhanced absorption of calcium from the intestinal lumen by vitamin D; PTH increases the formation of 1,25-$(OH)_2D_3$.

- The effects of persistent PTH or PTHRP activity on bone in the presence of a normal supply of vitamin D and calcium.

- ***Primary hyperparathyroidism***

Caused by inappropriate secretion of PTH by the parathyroid glands, causing hypercalcaemia. Due to one or more parathyroid adenomas, but occasionally to hyperplasia of all four parathyroid glands or to carcinoma of one of the glands. Ectopic parathyroid tumours do also occur. Primary hyperparathyroidism may be associated with other multiple endocrine neoplasias (MENs), such as pituitary and pancreatic adenomas (MEN type I), or with phaeochromocytomas and medullary carcinoma of the thyroid (MEN type II).

- The majority of cases of primary hyperthyroidism are diagnosed after finding of high plasma calcium, usually with low plasma phosphate concentrations.
- The clinical symptoms and signs at presentation, due to hypercalcaemia and include the following:

- ***Generalized ill health*** Depression, nausea, anorexia and abdominal pain and polyuria.
- ***Renal calculi*** About 10% of patients who present with renal calculi have primary hyperparathyroidism.

- ***Bone pain*** In most patients, subperiosteal bone erosions or cysts may be seen on radiography of the terminal phalanges. There are increased numbers of osteoclasts and an increased risk of bone fracture.
- ***Medical emergency*** Occasionally patients are admitted as an emergency with abdominal pain, vomiting and constipation.

- ***Tertiary hyperparathyroidism***

Occur if the parathyroid glands have been subjected to long-standing and sustained positive feedback by low plasma free ionized calcium concentrations (hypocalcaemia) of secondary hyperparathyroidism which have been subsequently corrected.

The parathyroid glands hypertrophy; PTH secretion becomes partly autonomous and is not suppressed by negative feedback by the hypercalcaemia.

The diagnosis is usually made when the cause of the original hypocalcaemia is removed, e.g. by renal transplantation or correction of long-standing calcium or vitamin D deficiency as in malabsorption. A history of previous hypocalcaemia and very high plasma ALP activity due to the prolonged osteomalacia distinguish it from primary hyperparathyroidism.

- **Hypercalcaemia of malignancy**

1. Malignant disease of bone

Multiple bony metastases (e.g. breast, lung, prostate, kidney and thyroid tumours) or with multiple myeloma show hypercalcaemia. Here there is usually a parallel rise of plasma phosphate. The hypercalcaemia is caused by direct bone breakdown due to the local action of malignant deposits and cytokine activation.

Hypercalcaemia of malignancy

2. Humoral hypercalcaemia of malignancy

PTHRP is synthesized by some malignant tumours of non-endocrine tissues and is not subject to normal feedback control by the high plasma free ionized calcium concentration.

Hypercalcaemia of malignancy

Plasma ALP activity may be raised because of secondary deposits in bone or the liver, or both. In humoral hypercalcaemia of malignancy, the plasma calcium concentration may rise to dangerously high very rapidly, in contrast to primary hyperparathyroidism.

- **Drugs/medications**

E.g. thiazides (decreases calcium renal excretion), lithium, and vitamin A excess.

- **Milk- alkali syndrome**

This rare condition occurs with the excessive use of calcium-containing antacids for dyspepsia.

- **Vitamin D excess**

Increased intestinal calcium absorption may cause dangerous hypercalcaemia.

- **Sarcoidosis**

Sarcoidosis: inflammatory disease, mostly in lymph glands, abnormal masses or nodules (called granulomas).

Hypercalcaemia is a rare complication. 1,25-Dihydroxycholecalciferol is synthesized in the granuloma tissue and increases calcium absorption from the intestinal tract.

- **Hypercalcaemia of hyperthyroidism**

Prolonged excess of thyroid hormone in severe hyperthyroidism may be associated with the histological appearance of osteoporosis and a consequent increase in urinary calcium excretion.

- **Other endocrine causes of hypercalcaemia**

Acromegaly, Addison's disease and phaeochromocytoma.

- **Familial hypocalciuric hypercalcaemmia**

Hypercalcaemia & inappropriately high plasma PTH concentration & hypocalciuria has been reported in some families. The condition is inherited as an autosomal dominant trait. The aetiology is thought to be due a defect on the CaSR. Low urinary calcium concentration in the face of hypercalcaemia points to the diagnosis.

A useful test is the calcium excreted per litre of glomerular filtrate (CaE):

$$\mathrm{CaE} = \frac{\mathrm{urinary[calcium]} \times \mathrm{plasma[creatinine]}}{\mathrm{urinary[creatinine]}}$$

Hypocalciuric hypercalcaemia is likely if this is less than 0.015 (mmol/L).

- **Hypercalcaemia of infancy**

Idiopathic hypercalcaemia of infancy includes a number of conditions that cause hypercalcaemia during the first year of life. Williams' syndrome is a rare familial disorder associated with increased intestinal calcium absorption and hypercalcaemia. Clinical features include growth retardation, mental deficiency and characteristic 'elfin' facies.

Investigation of hypercalcaemia

Two groups of causes should be differentiated for raised plasma albumin-adjusted calcium concentration:

- Raised albumin-adjusted calcium concentration due to inappropriately high PTH and usually hypophosphataemia.
- Raised albumin-adjusted calcium concentration due to other causes and associated with low PTH concentrations and often hyperphosphataemia.

Investigation of hypercalcaemia

The following procedure to find the cause of hypercalcaemia, all the steps have been followed:

- Establish the plasma albumin concentration.
- Check the albumin-adjusted calcium. Take a specimen without venous stasis (preferably without a tourniquet) to eliminate artefactual haemoconcentration and repeat the plasma calcium and albumin assays.

Investigation of hypercalcaemia

- Take a careful history, with special reference to the drug history, such as vitamin-D-containing preparations and thiazide diuretics. Is there evidence of milk–alkali syndrome, albeit rare now? If so, check acid–base status.
- Is the plasma phosphate concentration low in relation to the renal function? Hypophosphataemia suggests the diagnosis of primary hyperparathyroidism.

hypocalcaemia

- **Clinical effects of a reduced albumin-adjusted plasma calcium concentration**

- Increased neuromuscular activity eventually leading to tetany and carpopedal spasm, generalized seizures, laryngospasm, hyper-reflexia, paraesthesiae and hypotension.
- Prolonged hypocalcaemia may cause cataracts.
- Hypocalcaemia may also cause depression and other psychiatric symptoms as well as cardiac arrhythmias

Hypocalcaemia with hypophosphataemia

- **Reduced intake and absorption of calcium and vitamin D**

In steatorrhoea, fat (and therefore vitamin D) absorption is impaired; this malabsorption may be aggravated if calcium combines with unabsorbed fatty acids to form insoluble soaps in the lumen.

Hypocalcaemia with hypophosphataemia

- The following groups are at risk of developing osteomalacia or rickets:
  - children and pregnant women.
  - people such as the elderly and chronically sick who are less exposed to sunlight.

Hypocalcaemia with hypophosphataemia

- **Impaired metabolism of vitamin D**

✓ Chronic liver disease.

✓ Prolonged anticonvulsant therapy.

✓ Type 1 vitamin-D-dependent rickets is due to 1- α - hydroxylase deficiency. low 1,25-$(OH)_2D_3$ concentrations.

✓ Type 2 vitamin-D-dependent rickets is also an autosomal recessive disorder and causes a vitamin D resistance and is a defect of the vitamin D or calcitriol receptor. low 1,25-$(OH)_2D_3$ concentrations.

Hypocalcaemia with hyperphosphataemia

- **Renal dysfunction**

Chronic kidney disease causes relative resistance to vitamin D because of the direct effect of the disease on the functioning renal tubular cells and therefore inhibition of 1- α -hydroxylation by hyperphosphataemia associated with the low GFR of renal glomerular dysfunction.

Hypocalcaemia with hyperphosphataemia

- **Primary hypoparathyroidism**

✓ Accidental damage.

✓ Autoimmune hypoparathyroidism.

✓ Congenital absence of parathyroid gland, it is rare.

Hypocalcaemia with hyperphosphataemia

- **Pseudohypoparathyroidism**

✓ It is very rare inborn error associated with an impaired response of both kidneys and bone to PTH. Increase PTH with low calcium.

- **Secondary hyperparathyroidism**

Disorders of bone disease associated with secondary hyperparathyroidism may present:

✓ *With osteomalacia* in adults, or *rickets* in children

✓ *Without osteomalacia or rickets*:

Hypocalcaemia with hyperphosphataemia

- ***With osteomalacia* or *rickets* :**

Predisposing factors include:

- Reduced dietary intake of vitamin D, calcium and phosphate in undernutrition.

- Impaired absorption of vitamin D, e.g. in steatorrhoea or hepatobiliary disease.

- Impaired metabolism of vitamin D to 1,25- $(OH)_2$ D_3 due to renal disease.

- Increased inactivation of vitamin D due to anticonvulsant therapy.

- Renal tubular disorders of phosphate reabsorption.

Hypocalcaemia with hyperphosphataemia

- ***Without osteomalacia or rickets*:** if PTH action is inadequate to correct the abnormality, the plasma calcium concentration remains low and bone disorders are not present.
  Predisposing factors include:

– early calcium and vitamin D deficiency,

– the rare pseudohypoparathyroidism.

Investigation of hypocalcaemia

- hypocalcaemia fall into two main groups:
  - Reduced albumin-adjusted calcium concentration due to primary PTH deficiency and associated with hyperphosphataemia.
  - reduced albumin-adjusted calcium concentration due to other causes and associated with appropriately high PTH concentrations and usually hypophosphataemia.

Hypercalciuria

- Hypercalciuria in the absence of hypercalcaemia (hypercalciuria normocalcaemia) may predispose to the formation of renal calculi and may occur in:

1. some cases of osteoporosis in which calcium cannot be deposited in normal amounts because the bone matrix is reduced.
2. acidosis, in which the release of free ionized calcium from bone is increased.

Hypercalciuria

- Hypercalciuria divided into:

✓ absorptive hypercalciuria, type I (hyperabsorption of calcium), type II (diet-responsive hypercalciuria) and type III (renal phosphate leak resulting in decreased calcium resorption).

✓ renal hypercalciuria (decreased renal calcium resorption).

- Hypercalciuria can, of course, also occur in the face of hypercalcaemia, such as resorptive hypercalciuria associated with primary hyperparathyroidism.

Reference

- Crook, M., 2012. *Clinical biochemistry and metabolic medicine*. CRC Press.