Lecture 6 **Dr. Maytham Ahmed**

Calcium, Inorganic phosphate, Serum chloride

Calcium

Test description

Within the circulation, calcium is found in several different forms, with 46% free, 32% bound to albumin, 8% bound to globulins, and 14% associated in freely diffusible calcium complexes. The amount of calcium in blood is minute compared to the 98% to 99% present in the teeth and bone. It is essential for calcification of bones and teeth, blood clotting, muscle contractility, heart function and transmission of nerve impulses.

Parathyroid hormone, calcitonin hormone and vitamin D are important for calcium homeostasis. There is an inverse relationship between calcium and phosphorus: as serum calcium levels increase, serum phosphorus levels decrease.

Total calcium test in the blood measures the total calcium present in the blood. This provides information regarding parathyroid gland function and metabolism of calcium. It is also used to evaluate malignancies, since cancer cells release calcium, often resulting in high calcium levels in the blood (hypercalcemia).

Specimen

- No fasting is usually required prior to the test, although some laboratories do require a fast with water permitted.

- Obtain venous blood sample to get serum or plasma for calcium estimation. Heparinized plasma is preferred for ionized calcium studies.

- Serum or heparinized plasma is separated from cells as rapidly as possible to avoid the uptake of calcium by erythrocytes.

- EDTA or oxalate decrease calcium levels.

- Calcium oxide can leach from glass tubes as it is one of its components, so plastic tubes are used instead.

- Stasis changes concentration of protein bound calcium. Avoid tourniquet use (otherwise falsely high calcium levels).

- The fraction of calcium bound to protein in serum is pH-dependent. Acidosis increases the ionized fraction (decreases binding), whereas alkalosis increases the bound fraction.

- Serum or heparinized plasma samples may be stored at room temperature for up to 8 hours, at 4°C for 1 day, or frozen for up to 1 year. Calcium in specimens collected and stored in gel-separator tubes did not show any difference compared with specimens collected and stored in tubes not containing gel.

Interpretation

Total serum calcium concentration is influenced by (1) the serum albumin concentration, (2) position of the body when the calcium is drawn, and (3) tourniquet stasis. A decrease of serum albumin of 1 gram will decrease the total serum calcium by about 0.8 mg. Posture changes the serum calcium concentration; going from an upright to a recumbent posture causes a decrease of approximately 4% (range 2% to 7%). The ionized calcium is not influenced by these factors and is the better indicator of the hypercalcemic or hypocalcemic state. The ionized calcium may be calculated from total protein, albumin and calcium values, but direct measurement is preferable.

Interfering factors

- Increased (hypercalcemia) in: hyperparathyroidism, vitamin D in excess, multiple myeloma, leukemia, metastatic cancer in bones, Paget's disease, prolonged immobilization, excessive ingestion of milk, decrease in pH (acidosis), drug that my increase calcium include: calcium salt, hydralazine, thiazide diuretics, parathyroid hormone, thyroid hormones, calcium containing antacids, androgens, estrogens and progesterone.

- Decreased (hypocalcemia) in: hypoparathyroidism, vitamin D deficiency, renal insufficiency, hyperphosphatemia, acute pancreatitis, malabsorption, osteoporosis, osteomalacia, rickets, metabolic alkalosis, hypoalbuminemia artifactually associated with decreased levels of total calcium, drugs that my decrease calcium include: aspirin, corticosteroids, heparin, laxatives, anticonvulsants, loop diuretics, magnesium salts.

Normal level of total calcium

8.5-10.5 mg/dL (2.1-2.6) mmol/L SI units)

In elderly the level of total calcium decreased.

Conversion formula for converting mmol/L to mg/dL in plasma specimen: **Multiply mmol/L by the number 4**

Detection methods

1- Colorimetric method: provides a simple assay to determine calcium. Its principle depend on the formation of chromogenic complex between calcium ions and o-cresolphthalein.

Direct spectrophotometric measurements of calcium in serum or urine are based upon formation of color complexes between calcium and organic molecules. Of these calcium-complexing, colorimetric reagents, the o-cresolphthalein and arsenazo III dye are the most commonly used for routine calcium analysis.

2- Atomic absorption spectrophotometry.

Inorganic phosphate

Test description

Most of the body's phosphorus is combined with calcium in bones. About 15%, however, exists in the blood, making the phosphorus the main anion in the intracellular fluid. It has several functions, including a role in glucose and lipid metabolism, storage and transfer of energy within the body, generation of bony tissue, and maintenance of acid-base balance. Like calcium, phosphorus is controlled by the parathyroid hormone (PTH). It holds an inverse relationship with calcium; an excess in the serum of one results in the kidneys excreting the other. The PTH increases calcium and phosphate release from bone and decreases loss of calcium and increases loss of phosphate in the urine. In the body, phosphorus is combined with oxygen to form a variety of phosphates (PO_4^{3-}). An elevated serum phosphorus level is known as hyperphosphatemia; decreased level hypophosphatemia.

The **phosphorus blood test** measures the amount of phosphate in the blood.

Specimen

- Plasma, serum can be used.

- Serum phosphate should ideally be determined in the fasting state. A recent meal, high glucose ingestion, insulin release or administration, muscular activity, and hyperventilation can all lower serum phosphate by causing a shift from the plasma into the cells.

The evidence for practice

Serum levels of calcium, phosphorus, alkaline phosphatase, total carbon dioxide (CO_2) and PTH should be measured in all patients with chronic kidney disease (CKD) Stages 2 through 5. The frequency of these measurements should be based on the stages of CKD.

Clinical alerts

Serum phosphorus should be correlated with serum calcium levels to determine possible causes

(1) Increased phosphorus with decreased calcium: hypoparathyroidism, renal disease.

(2) Increased phosphorus with normal or increased calcium: milk-alkali syndrome, hypervitaminosis D.

(3) Decreased phosphorus with increased calcium: hyperparathyroidism, sarcoidosis.

(4) Decreased phosphorus and calcium: malabsorption, vitamin D deficiency, renal tubular acidosis.

Normal values

2.4 - 4.1 mg/dL (0.78-1.34 mmol/L SI units), this fluctuates with age (it is higher in children than adults)

Conversion formula for converting mmol/L to mg/dL: **Multiply mmol/L by the number 3**

Conditions that increase or decrease phosphate level

Increased (hyperphosphatemia) in: acromegaly, bone tumors, healing fracture, hypocalcemia, hypoparathyroidism, phosphate supplementation, prepuberty, renal failure, vitamin D intoxication.

Decreased in (hypophosphatemia): antacid abuse, carbohydrate loading, diuresis, hypercalcemia, hyperparathyroidism, malabsorption, malnutrition, rickets, vitamin D deficiency.

Contributing factors to abnormal values

(1) Hemolysis of the blood sample may alter test results.

(2) Use of tourniquet during acquisition of the blood sample may alter test results.

(3) Due to increased carbohydrate metabolism causing decreased phosphorus levels, glucose solutions should not be infused prior to the test.

(4) Examples of drugs which may increase serum phosphate level: phosphate enemas, risedronate, and vitamin D.

(5) Examples of drugs which may decrease serum phosphate level: anticonvulsants, azathioprine, calcitriol, insulin, IV dextrose, lithium, phosphate binding agents.

Techniques for the determination of phosphorus

Serum inorganic phosphate is measured either colorimetrically or isotopically; the latter is not in routine use.

CHLORIDE

Test description

It is the major anion of the extracellular fluid. Chloride has several functions, including maintaining electrical neutrality, act as one component of the buffering system, aiding in digestion, and helping to maintain osmotic pressure and water balance.

Because chloride is most often seen in combination with sodium, shifts in sodium levels result in corresponding shifts in chloride levels.

Blood chloride testing is often evaluated as part of screening laboratory tests. It may also be ordered to evaluate patients complaining of prolonged vomiting or diarrhea or weakness.

Chloride levels have an inverse relationship with those of bicarbonate; thus, they reflect acid base status. When chloride as HCl or NH_4Cl is lost, alkalosis is obtained. When chloride is retained or ingested, acidosis is obtained.

Specimen

- Plasma, serum can be used.

- No fasting is required prior to the test.

Normal value

96-106 mEq/ L (96-106 mmol/L SI units)

Conditions that increase or decrease chloride level

Increased (hyperchloremia**) in**: renal insufficiency, dehydration, overtreatment with saline infusion, and Cushing's syndrome.

Decreased (hypochloremia) **in**: vomiting, diarrhea, excessive sweating, and adrenal insufficiency.

Techniques for the determination of chloride

Several techniques for chloride determination are available including:

(1) The autoanalyzer (a colorimetric technique)

(2) A coulometric method

(3) A mercurimetric method

(4) Chloride-specific ion electrodes.

In the autoanalyzer method, chloride ions displace thiocyanate from mercuric thiocyanate. The free thiocyanate reacts with ferric ions to form a colored complex, ferric thiocyanate, which is measured photometrically.

Contributing factors to abnormal values

(1) Hemolysis of the blood sample may alter test results.

(2) Use of tourniquet during acquisition of the blood sample may alter test results.

(3) Examples of drugs which may increase serum chloride: ammonium chloride, and sodium chloride.

(4) Examples of drugs which may decrease serum chloride: ethacrynic acid, loop diuretics