Serum Alkaline Phosphatase and Related Clinical Cases

Note: The objective of this document is to give a brief overview about how to estimate serum level of alkaline phosphatase (ALP). The topic will be taken up in detail during the practical session. After going through the related clinical cases, you should be able to identify the conditions/diseases in which its levels are raised.

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Phosphatases are hydrolases. They hydrolyze a variety of organic phosphate esters transferring phosphate groups from a donor substrate to an acceptor containing a hydroxyl group. The active centre of these enzymes contains a serine residue. Two types are commonly estimated in serum:

- A mixture of alkaline phosphatases with maximum activity at pH 10.
- Acid phosphatases with maximum activity between pH 5-6.

Alkaline phosphatases (ALPs) are a family of isoenzymes. They are membrane-bound glycoproteins and zinc containing metalloenzymes.

Tissue Distribution: They are present practically in all tissues of the body. High levels are present in:

- Intestine: The small intestinal epithelium (I).
- Liver: The bile canalicular and sinusoidal membrane of the liver (L)
- Bone: The osteoblasts in the bone, especially in infancy and childhood when there is active bone growth (B).
- Kidney: The tubular epithelium of the kidney (K).
- The placenta and the lactating breast (P).

Characterization and differentiation of the ALPs: Many different biochemical and immunological methods have been used to differentiate between and selectively assay the different ALPS at the enzyme and protein level.

Three general methods have proved particularly useful:

1. Thermostability studies
2. Differential inhibition with various amino acids, small peptides and other low molecular weight substances
3. Immunological methods

The intestinal and L/B/K ALPs are rapidly inactivated at temperature 65 ^{o}C.

In contrast, placental and placental-like ALPS are remarkably thermostable. They may be heated at 65 ^{o}C for an hour or more without loss of activity.

However, the intestinal ALP is somewhat more thermostable than the L/B/K ALP. It has also been shown that in the serum, ***liver ALP is slightly, though significantly, more thermostable than bone ALP***.

Points to remember: Alkaline phosphatases are a group of true isoenzymes, encoded for by at least four different genes: tissue-nonspecific, intestinal, placental, and germ-cell ALP. The

isoforms derived from the tissue-nonspecific isoenzyme by post-translational modification include the variants of the enzyme found in the liver, bone, kidney and in the placenta in the first trimester of pregnancy. Some malignant tumours can produce a placental form of the enzyme called the Regan's isoenzyme. The various forms of the enzyme originating from the different tissues can be separated by electrophoresis on starch, cellulose acetate or polyacrylamide gels.

The **enzyme unit**, or international **unit** for **enzyme** (symbol U, sometimes also IU) is a **unit of enzyme**'s catalytic **activity**. 1 U (µmol/min) is **defined** as the amount of the **enzyme** that catalyzes the conversion of **one** micromole of substrate per minute under the specified conditions of the assay method.

We measure the enzyme activity of alkaline phosphatase, in this particular estimation, rather than the amount of enzyme present (which is miniscule, so its estimation would pose a problem).

METHODS OF ESTIMATION:

- **King and King method**
- King and Armstrong method
- Bessey, Lowry and Brock method
- Bodansky method

PRINCIPLE:

Phenol released by enzymatic hydrolysis from disodium phenylphosphate under defined conditions of time, temperature and pH, is estimated colorimetrically (**By King & King Method**)

$$\text{Phenyl phosphate} \xrightarrow[\text{Alk.PO4ase; pH 10}]{37^{o}C,\ \text{Serum};\ Mg^{2+}} \text{Phenol} + \text{Phosphate}$$

The phenol liberated reacts with 4-amino antipyrine (4-amino phenazone) in the presence of alkaline oxidizing agent (potassium ferricyanide) to give a purple or red colored complex (potassium ferricyanide enhances the color).

Reagents:

1. **Bicarbonate Buffer** (pH 10.0)
2. **Substrate:** 0.01 M-disodium phenyl phosphate
3. **Working Phenol Standard:** (1mg/100ml).
4. **0.5 N-Sodium hydroxide**
5. **0.5 N-Sodium bicarbonate**
6. **4-Aminoantipyrine**
7. **Potassium ferricyanide**.

PROCEDURE Set up 4 tubes as follows:

Pipette into tubes Marked	Test	Control	Standard	Blank
Substrate	1ml	1ml	-	-
Bicarbonate Buffer (for maintaining alkaline pH)	1 ml	1ml	1.1ml	1.1ml
Pre-Incubate (to bring all reactants at 37°C) the test tubes in water bath at 37^0C for 3 min				
Serum	0.1ml	-	-	-
Phenol Standard(10µg/ml)	-	-	1ml	-
Distilled water	-	-	-	1ml
Incubate the test tubes in water bath at 37^0C for 15 min				
0.5 N-NaOH (to stop reaction by making pH highly alkaline)	0.8ml	0.8ml	0.8ml	0.8ml
Serum	-	0.1ml	-	-
$NaHCO_3$ (makes the pH 10)	1.2ml	1.2ml	1.2ml	1.2ml
4-Aminoantipyrine	1ml	1ml	1ml	1ml
Potassium ferricyanide (oxidizing agent to potentiate colour development)	1ml	1ml	1ml	1ml

It is important to mix thoroughly because failure to do so leads to erroneous result. The reddish purple colour that develops should be read immediately at 510 mm or with a green filter.

Why do we take a Control tube??

In the set- up of the control tube, all reactants and conditions are present, but the reaction does not take place. It is used to negate the contribution of other substances (other phenolic compounds already present in serum sample) to the final colour of the solution. (Note that phenolic compounds in sera also give colour – this may lead to a false depth of the colour obtained)

CALCULATIONS:

CALCULATION (WILL BE DISCUSSED DURING PRACTICAL SESSION)

$$Serum\ ALP\ activity = \frac{OD\ of\ Test - OD\ of\ Control}{OD\ of\ Standard - OD\ of\ Blank}\ x\ Conc.of\ Standard$$

$$Sr.ALP\ activity = Amount\ of\ phenol\ librated\ in\ the\ reaction\ by\ ALP\ in\ Serum.$$

INTERPRETATION:

Reference Range: Normal serum level of alkaline phosphates in adults is ***3-13 KA units/dl (35-94IU/L).***

Clinical Significance:

Causes of raised levels are:

1.Physiological causes:

- In children, due to active bone growth (1.2-2.5 times the normal adult values)
- In late pregnancy and parturition (placental origin). There may be 2-3-fold increase in the third trimester

2.Pathological causes: Elevated levels in disease almost entirely conform either to bone diseases or to diseases of the hepatobiliary system.

The bone diseases include diseases with increased osteoblastic activity.

Highest levels are encountered in ***Paget's disease*** (10-20 times).

In ***rickets and osteomalacia***, moderate increase in level is observed.

Other causes include hyperparathyroidism, osteogenic bone cancers, healing phase of fractures (transient elevation). In osteolytic bone diseases for example in multiple myeloma the serum levels are normal.

Hepatobiliary causes include extra-hepatic or intra-hepatic biliary obstruction. In extrahepatic obstruction like stones and cancer of the head of the pancreas, a marked increase is seen.

In intrahepatic obstruction like metastasis to liver, biliary cirrhosis, cholestatic liver damage due to certain drugs like chlorpromazine, moderate increase is seen.

In acute hepatitis there may be slight increase (up to 1.5-2 folds).

Note: The serum level of **5' Nucleotidase** is elevated in obstructive biliary disease but is unaltered in bone disease, and this may help to distinguish between the two conditions.

CLINICAL CASES: PROBLEM BASED LEARNING

INSTRUCTIONS: PREPARE THE ANSWERS TO THE QUESTIONS FOR THE FOLLOWING CASES

Case 1

A 65-year-old retired man presented with complaints of upper abdominal pain, anorexia, generalized pruritis and weight loss. He was observed to have jaundice and gave history of passing of high colored urine and clay colored, frothy and sticky stools. Investigations revealed the diagnosis of malignancy of the head of pancreas. The patient was taken up for surgery. His laboratory investigations revealed the following:

- S. Bilirubin – 9.0 mg/dl
- Total proteins — 7.3gm/dl
- Albumin -- 4.1 gm/dl
- ALP -- 510 IU/L
- AST – 80 IU/L
- ALT – 85IU/L

QUESTIONS

a. What is the type of bilirubinemia you would expect to see in this case?

b. What are the abnormalities in the urine?

c. What is the cause of increased alkaline phosphatase (ALP) in this case? What are the tissues rich in ALP? Under which normal circumstances do you expect ALP to be raised?

d. What is the cause of the changed characteristics of the stools?

e. What is the cause of the pruritis in this patient?

f. What may be the change of Prothrombin Time of this patient and why?

Case 2

A 65-year-old male patient underwent trans-urethral prostatic resection (TURP) for benign hypertrophy of prostate. Laboratory reports revealed progressive increase in serum ALP (950 U/L). Bone scan had revealed multiple hot spots. Urosurgeon had ruled out Prostatic metastasis. A diagnosis of Paget's disease was made and treatment given. He was reviewed few years later with his symptoms worsened and Serum Alkaline phosphatase had increased to 1720 U/L.

Questions

a. What was the reason for rise in serum ALP activity in this patient?
b. What are the various tissue sources of ALP?
c. How can you differentiate between Bone and Liver Specific ALP?
d. What is the difference between an isoform and isoenzyme?
e. What is Regan isoenzyme and what is its importance?