Clinical Enzymology (plasma enzyme in diagnosis)

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• Most enzymes are present in cells at much higher concentrations than in plasma.
• Some occur predominantly in cells of certain tissues, where they may be located in different cellular compartments such as the cytoplasm or the mitochondria.
• 'Normal' plasma enzyme levels reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation
• The enzyme activity in plasma may be:
• Increased due to proliferation of cells, an increase in the rate of cell turnover or damage or in enzyme synthesis (induction), or to reduced clearance from plasma.
• Lower than normal, very occasionally due to reduced synthesis or congenital deficiency. Changes in plasma enzyme activities may sometimes help to detect and localize tissue cell damage or proliferation, or to monitor treatment and progress of disease.
Assessment of Cell Damage and Proliferation

• Plasma enzyme levels depend on:
  1. The rate of release from damaged cells which, in turn, depends on the rate which damage is occurring:
  2. The extent of cell damage. In the absence of cell damage the rate release depends on:
     1. The rate of cell proliferation.
     2. The degree of induction of enzyme synthesis

These factors are balanced by:

• The rate of enzyme clearance from circulation.
• It is not known how most enzymes are removed from, or their action inhibited in the circulation.
• Relatively small peptides, such as α-amylase, can be cleared by the kidneys.
• Most enzymes are large proteins and are probably catabolized by plasma proteases before being taken up by the reticuloendothelial system.
• In health each enzyme has a fairly constant and characteristic biological half-life; knowledge of this half-life may be of help in assessing the time since the onset of an acute illness.
• After a myocardial infarction, for example, plasma levels of creatine kinase and aspartate transaminase fall to normal before those of lactate dehydrogenase, which has a longer half-life. The half-life may be lengthened if there is circulatory impairment.

• Measurement of the plasma activity of an enzyme known to be in high concentration within cells of a particular tissue may indicate an abnormality of those cells, but the results will rarely enable a specific diagnosis to be made.
• For example if there is circulatory failure after a cardiac arrest very high plasma levels of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance:
• the raised plasma levels of 'cardiac' enzymes do not necessarily mean that a myocardial infarct caused the arrest.
• The diagnostic precision of plasma enzyme analysis may be improved by:

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1. Estimation of more than one enzyme. Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For instance, although both alanine and aspartate transaminases are abundant in the liver, the concentration of aspartate transaminase is much greater than that of alanine transaminase in heart muscle.

2. Isoenzyme determination. Some enzymes exist in more than one form: these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues such identification will give more information than the measurement of plasma total enzyme activity: for example, creatine kinase may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium.
3. Serial enzyme estimations. The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or occasionally of impaired clearance. The distribution of enzymes within cells may differ. Alanine transaminase and lactate dehydrogenase are predominantly located in cytoplasm and glutamate dehydrogenase in mitochondria, whereas aspartate transaminase occurs in both these cellular compartments.
Factors Affecting Results of Plasma Enzyme Assays:

1-Analytical factors affecting results. The total concentration of all plasma enzyme proteins is less than 1 g/L. Results of enzyme assays are not usually expressed as concentrations, but as activities. These include the concentrations of the substrate and product, the pH and temperature at which the reaction is carried out. The type of buffer, and the presence of activators or inhibitors.
2-Physiological factors affecting enzyme activities include for example: Age, Sex, physiological conditions: Plasma alkaline phosphatase activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme: several enzymes, such as the transaminases and creatine kinase rise moderately in plasma during and immediately after labour or strenuous exercise. Plasma enzyme activities must be interpreted in relation to the sex and age-matched reference ranges of the issuing laboratory.
Phenylketonuria: is an autosomal recessive disorder caused by an abnormality of the phenylalanine hydroxylase. Because phenylalanine cannot be converted to tyrosine it accumulates in plasma and is secreted in the urine with its metabolites, such as phenylpyruvic acid; the disease acquired its name from the detection of the latter "phenylketone" in the urine.

Alkaptonuria: is an autosomal recessive disorder due to the deficiency of homogentisic acid oxidase. Homogentisic acid accumulates in tissue and blood and is passed in the urine. Oxidation and polymerization of homogentisic acid produces the pigment alkapton in much the same way as polymerization of DOPA produce melanin.
3- glycogen storage disease: a deficiency of one of the enzymes involved in the glycogenesis or glycogenolysis results in the accumulation of normal or abnormal glycogen with hepatomegaly: in von Gierk's disease, the least rare glycogen storage disorder, there is a deficiency of glucose-6-phosphate. Fasting hypoglycemia occurs because the enzyme is essential for the conversion of glucose-6-phosphate to glucose.

4. galactosaemia an autosomal recessive disorder due to a deficiency of galactose-1-phosphate uridyltransferase, may cause cirrhosis of the liver if untreated.

5. cystinuria: is an autosomal recessive inherited abnormality of tubular reabsorption, with excessive urinary excretion of the dibasic amino acids: cystine, arginine and lysine.
Pancreatic enzymes:

**Acute pancreatitis** is an inflammatory process in which pancreatic enzymes are activated and cause autodigestion of the gland. It is a result of anatomical changes that arise from two events.

1. The first is the autodigestion of the acinar cells by inappropriate activation of the pancreatic enzymes (especially trypsinogen) within the cell.
2. The second is the cellular injury response that is mediated by proinflammatory cytokines. The mechanisms by which the digestive enzymes become activated within the acinar cell are unclear. However, such inappropriate activation of pancreatic enzymes leads to destruction of the acinar cell and surrounding fat deposits, and it weakens the elastic fibers of the blood vessels, resulting in leakage. There are some enzymes that are synthesized and stored as the active enzymes in the zymogen granules. These include α-amylase, carboxyl ester lipase, lipase, colipase, RNase, and DNase.
Pancreatic pseudocyst. If the plasma amylase activity fails to fall after an attack of acute pancreatitis there may be leakage of pancreatic fluid into the lesser sac (a pancreatic pseudocyst). Urinary amylase levels are high, differentiating it from macroamylasaemia. This is one of the few indications for estimating urinary amylase activity, which is inappropriately low relative to the plasma activity if there is glomerular impairment or macroamylasaemia.

Macroamylasaemia. In some patients a high plasma amylase activity is due to a low renal excretion of the enzyme, despite normal glomerular function. The condition is symptomless; it is thought that either the enzyme is bound to a high molecular weight plasma component such as protein, or that the amylase molecules form large polymers that cannot pass through the glomerular membrane. This harmless condition may be confused with other causes of hyperamylasaemia.
Liver enzymes:

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various heptobility disorders. There are three types of enzymes:

1. Enzymes which are normally present inside the hepatocytes released into the blood when there is a hepatocellular damage = **markers of hepatocellular damage**.

2. Enzymes which are primary membrane bound (plasma membrane or side of hepatocytes) = **markers of cholestasis**.

3. Enzymes which are synthesized in the hepatocyte = **indicates disturbances in the hepatocellular synthesis**.
Markers of hepatocellular damage.

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\text{L-Aspartate} + \text{2-Oxoglutarate} \xrightleftharpoons{\text{AST, P-5'-P}} \text{Oxaloacetate} \quad + \quad \text{L-Glutamate}
\]

\[
\text{L-Alanine} + \text{2-Oxoglutarate} \xrightleftharpoons{\text{ALT, P-5'-P}} \text{Pyruvate} \quad + \quad \text{L-Glutamate}
\]
o Location:
o AST present in cytosol and mitochondria
o ALT located in cytosol of liver
o In the liver, the concentration of ALT per unit weight of the tissue is more than AST.
o These enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.
o The highest activities of ALT are found in hepatocytes and muscle cells.
o Again the hepatocytes have very high activity of ALT. Therefore elevations in serum ALT are considered to be relatively specific for liver disease.

o In liver disease, the ALT level is increased markedly compared to AST.

o In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST
CAUSES OF RAISED PLASMA ALT ACTIVITIES

• Marked increase (10 to 100 times the upper limit of the adult reference range circulatory failure with 'shock' and hypoxia: Acute viral or toxic hepatitis.

• Moderate increase: Cirrhosis (may be normal or up to twice the upper adult reference limit): infectious mononucleosis (due to liver involvement):

• Liver congestion secondary to congestive cardiac failure: cholestatic jaundice (up to 10 times the upper reference limit in adults); surgery or extensive trauma and skeletal muscle disease (much less affected than AST)
2. Markers of cholestasis

. Alkaline phosphatase (Half-life= 10 days)

The alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in most tissues but are in particularly high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. The exact metabolic function of ALP is unknown but it is probably important for calcification of bone. In adults plasma ALP is derived mainly from bone and liver in approximately equal proportions: the proportion due to the bone fraction is increased when there is increased osteoblastic activity that may be physiological.
• Causes of raised Plasma ALP activity

• Physiological: There is a gradual increase in the proportion of liver ALP with age: in the elderly the plasma bone isoenzyme activity may increase slightly.
• · Bone disease rickets and osteomalacia secondary hyperparathyroidism.
• · Liver disease.
• · Malignancy. bone or liver involvement or direct tumor production.

• POSSIBLE CAUSES OF LOW PLASMA ALP ACTIVITY
• · Arrested bone growth
• · Hypophosphatasia: an autosomal recessive disorder, associated with rickets or osteomalacia.
ISOENZYMES OF ALKALINE PHOSPHATASE

• Bone disease with increased osteoblastic activity, or liver disease with involvement of the biliary tracts, are the commonest causes of an increased total alkaline phosphatase activity. Rarely the cause is not apparent and further tests may be helpful. The isoenzymes originating from cells of bone, liver, intestine and placenta may be separated by electrophoresis, but interpretation may be difficult if the total activity is only marginally raised. Assays for ALP isoenzymes are needed when:
• I. The source of an elevated ALP in serum is not obvious and should be clarified.
• II. The main clinical question is concerned with detecting the presence of liver or bone involvement
• III. In the case of metabolic bone disorders, to ascertain any modifications in the activity of osteblastes to monitor the disease activity and the effect of appropriate therapies.
2. Gamma-glutamyl-transferase
catalyzes the transfer of the γ-glutamyl group from peptides and compounds that contain it to an acceptor. Gamma-glutamyltransferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

• Other enzymes
• 1. Cholinesterase which is called true cholinesterase or choline esterase I.
  found in:
  • a. erythrocytes
  • b. lung and spleen
  • c. nerve endings
  • d. the gray matter of the brain.
• The second cholinesterase is **acylcholine acylhydrolase**
  It is also called: (1) pseudocholinesterase, (2) serum cholinesterase, (3) butyryl cholinesterase, (4) choline esterase II
• Although it is found in the:
  • 1. liver
  • 2. pancreas
  • 3. heart
  • 4. white matter of the brain
  • 5. serum
  Its biological role is unknown
• **Causes of decreased plasma cholinesterase activity**
  • Hepatic parenchymal disease (reduced synthesis).
  • Ingestion or absorption through the skin, of such anticholinesterases as organophosphates.
  • Inherited abnormal cholinesterase variants, with low biological activity.
• **Causes of increased plasma cholinesterase activity**
  • a. Recovery from liver damage (actively growing hepatocytes)
  • b. Nephrotic syndrome
• 2. **Glutamate dehydrogenase (EC 1.4.1.3; L-glutamate: NAD(P)+ oxidoreductase, deaminating; GLD)** is a mitochondrial enzyme found mainly in the:
  • a. liver
  • b. heart muscle
  • c. kidneys but small amounts occur in other tissue, including
  • d. brain
  • e. skeletal muscle tissue
  • f. leukocytes
Muscle enzymes:

1. Creatine Kinase

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\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatinine} + \text{ATP}
\]

\[
\text{ATP} + \text{glucose} \xrightarrow{\text{HK}} \text{glucose-6-phosphate} + \text{ADP}
\]

\[
\text{Glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G6PD}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+}
\]

CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle. The concentration gradients between some human tissues and serum for creatin kinase.
2. **Lactate Dehydrogenase**
catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity is therefore a non-specific marker of cell damage.
CAUSES OF RAISED PLASMA TOTAL LD ACTIVITY

• Artefactual: Due to in vitro haemolysis or delayed separation of plasma from whole blood.

• Marked increase (more than 5 times the upper reference limit in adults):

• Circulatory failure with 'shock' and hypoxia:

• Myocardial infarction

• Some haematological disorders. In blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas. very high levels (up to 20 times the upper reference limit in adults) may be found. Smaller increases occur in other disorders of erythropoiesis such as thalassaemia. myelofibrosis and haemolytic anaemias

• Renal infarction, or occasionally during rejection of a renal transplant. Moderate increase. viral hepatitis: malignancy of any tissue: skeletal muscle disease: pulmonary embolism: infectious mononucleosis.
ISOENZYMES OF LD

• LD1 fraction predominates in cells of cardiac muscle, erythrocytes and kidneys.
• LD5 is the most abundant form in the liver and in skeletal muscle. Whereas in many conditions there is an increase in all fractions; the finding of certain patterns is of diagnostic value.
• Predominant elevation of LD1 and LD5. (LD1 greater than LD5 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.
• Predominant elevation of LD2 and LD3 occurs in acute leukaemia: LD3 is the main isoenzyme elevated due to malignancy of many tissues.
• Elevation of LD5 occurs after damage to the liver or skeletal muscle.
Other clinically important enzymes

1. **Acid Phosphatase**: Acid phosphatase is present in lysosomes, which are organelles present in all cells with the possible exception of erythrocytes. Extralysosomal ACPs are also present in many cells:
   a. prostate,
   b. bone (osteoclasts),
   c. spleen
   d. platelets
   e. erythrocytes.

2. **Glucose-6-phosphate Dehydrogenase**
   is expressed in all cells and catalyzes the first step in the hexose monophosphate pathway, the conversion of glucose-6-phosphate to 6-phosphogluconate, generating NADPH.