CLINICAL ENZYMOLOGY

INTRODUCTION

Enzymes are catalysts that increase the rate or velocity of physiologic reactions. Each and every reaction in our body takes place with the help of an enzyme. In general, most enzymes are present in cells at much higher concentrations than in plasma. Measurement of their levels in plasma indicates whether their tissue of origin is damaged leading to the release of intracellular components into the blood. This forms the basis of clinical enzymology. Thus clinical enzymology refers to measurement of enzyme activity for the diagnosis and treatment of diseases.

PLASMA ENZYMES

Enzymes present in plasma can be classified into 2 types, they are

1- Functional Plasma enzymes

- Present in plasma at higher concentration than tissues
- They function in plasma.
- Mostly synthesized by the liver
- Usually decreased in disease conditions
- Eg. Clotting enzymes, lipoprotein lipase

2- Non-functional plasma enzymes

- Present in plasma at lower concentration than tissues
- Do not have any function in plasma
- Mostly synthesized by liver, skeletal muscle, heart, brain etc
- Usually increased in disease conditions
- Eg. Creatine kinase, Alanine transaminase etc
- Measurement of these enzymes in plasma can be used to assess cell damage and proliferation i.e. diagnosis of disease.

Assessment of Cell Damage and Proliferation

Plasma enzyme activities can be used in the diagnosis of disease and prognosis of treatment.

Plasma enzyme levels depend on balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood. The rate of influx is determined by the rate of release from damaged cells and altered rate of enzyme synthesis.

Localization of Damage

Enzymes used to measure tissue damage are present in nearly all cells with varying concentration. So the measurement may indicate an abnormality, but the specific diagnosis cannot 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

1. Estimation of more than one enzyme. Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For eg. 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. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities

Isoenzymes

Isoenzymes (also known as isozymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction

- Believed to be originating from closely linked genes or from multiple gene
- Evolution from a single form possibly due to long-term mutations
- They vary with respect to their kinetic parameters, electrophoretic mobility, and localization
- They all have independent action
- Eg.Lactate dehydrogenase have 5 isoenzymes (LDH1, LDH2, LDH3, LDH4 & LDH5)
- They can be used to identify the specific affected tissues
- They can be differentiated from each other and can be clinically quantified in the lab.

ENZYMES IN HEALTH AND DISEASES

Estimation of enzymes activities in the serum has many applications in the diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased), assessing prognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before the occurrence of jaundice). Some important enzymes of clinical significances are discussed below:

Distribution and application of clinically important enzymes

Enzymes	Tissues	Clinical applications
Alanineamino transferase	Liver	Hepato parenchymal diseases
Alkaline phosphatase	Liver, bone, intestinal mucosa, Placenta	Liver and bone diseases

Amylase	Salivary glands,	Pancreatic diseases
Aspartate amino transferase	Liver, Skeletal muscle, Heart, Erythrocytes	Hepatic parenchymal disease,Muscle disease
Cholinesterase	Liver	Organophosphorus insecticide poisoning, Hepatic parenchymal diseases
Creatine kinase	Skeletal muscle,Heart	Muscle diseases
Gamma glutamyl transferase	Liver	Hepatobiliary diseases, Marker of alcohol abuse
Lipase	Pancreas	Pancreatic diseases
Lactate dehydrogenase	Heart, liver, skeletal muscle erythrocytes, lymph nodes, Platelets	Hepatic parenchymal diseases,muscle diseases Hemolysis,tumor marker
5'nucleotidase	Liver	Hepatobiliary diseases
Trypsin	Pancreas	Pancreatic diseases

A: Pancreatic enzymes

1. α-Amylase:

belongs to hydrolyase class that catalyzes the hydrolysis of 1,4- a-glycosidic linkages in polysaccharides. They are low molecular weight proteins (54 to 62 kDa) that can pass the glomeruli of the kidneys. It is the only plasma enzyme physiologically found in urine. The AMY activity present in normal serum and urine is of pancreatic (P-AMY) and salivary gland (S-AMY)origin.

Clinical Significance

Normal values of amylase: 28-100 U/L = 0.48-1.7 ì kat/L

Causes of Raised Plasma Amylase Activity

1. Marked increase (five to 10 times the upper reference limit):

- Acute pancreatitis
- Severe glomerular impairment

2. Moderate increase (up to five times the upper reference limit):

- Perforated peptic ulcer

- Acute cholecystitis
- Intestinal obstruction
- Salivary gland disorders like mumps, salivary calculi

2. Lipase:

is a single–chain glycoprotein with molecular weight of 48 kDa.

Clinical Significance

Normal values: 40-200 U/L

- Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas.
- serum amylase is increased in mumps, pancreatic disease or due to some other cause, whereas lipase is increased only in pancreatitis. Therefore, the determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

3. Trypsin:

proteinase that hydrolyze the peptide bonds formed by the carboxyl groups of lysine arginine with other amino acids.

Clinical Significance

Normal values of trypsin: 25 ± 5.3 μ g/L

Increased in pancreatic disease. But as there is no distinct role of trypsin estimation in the routine management of patients with acute pancreatitis, this test is therefore considered of limited clinical value.

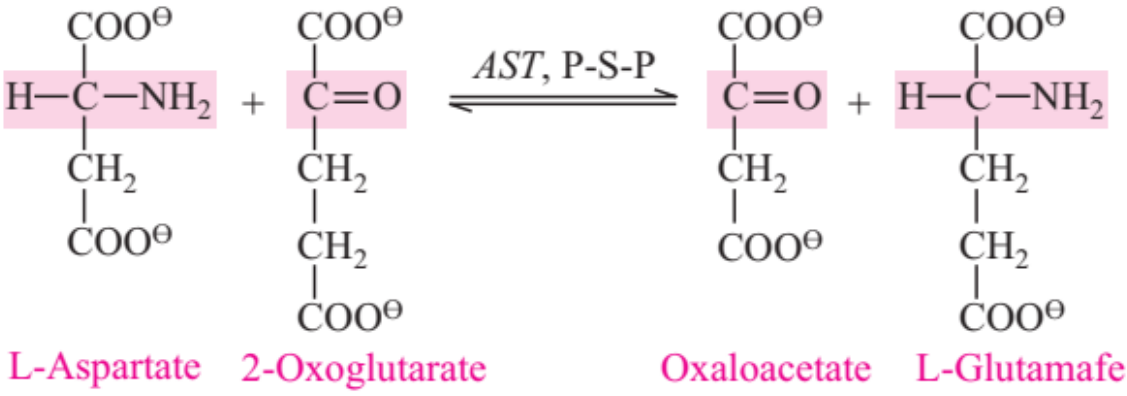
B: Liver enzymes

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various heptobiliy disorders.

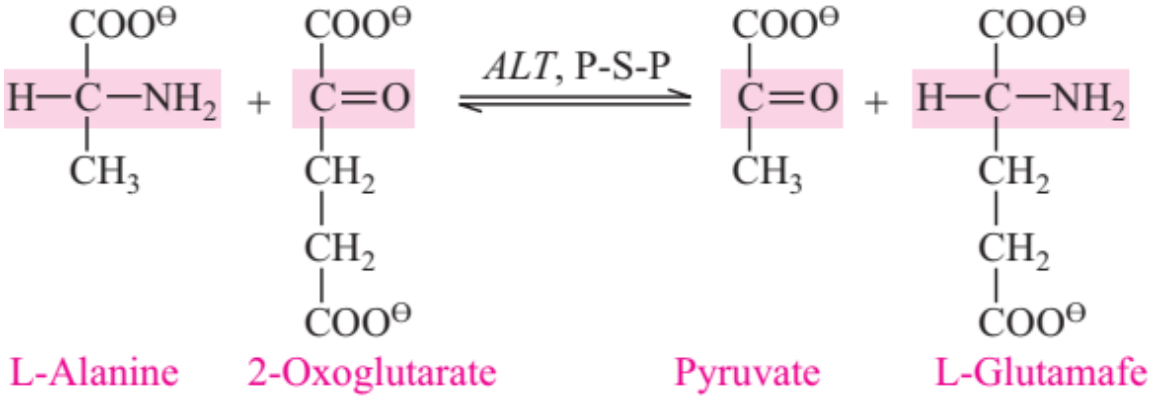
- **Markers of hepatocellular damage**

1. Aminotransferases/Transaminases

The transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid: they need the cofactor, pyridoxal phosphate for optimal activity. They are widely distributed in the body. The 2-oxoglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus AST catalyzes the reaction:



ALT catalyzes the analogous reaction:



The reactions are reversible, but the equilibrium of AST and ALT reactions favor formation of aspartate and alanine respectively.

In the liver, the concentration of ALT per unit weight of the tissue is more than AST.

- AST and ALT enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.
- Elevated plasma ALT are considered to be relatively specific for liver disease.
- AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.
- In liver disease, the ALT level is increased markedly compared to AST.

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.

(a) Aspartate Transaminase

Clinical Significance

Normal values of AST:
Male: <35 U/L = <0.60 mkat/L
Female: <31 U/L = <0.53 mkat/L

(b) Alanine Transaminase

Clinical Significance

Normal values of ALT:
Male: <45 U/L = <0.77 mkat/L
Female: <34 U/L = <0.58 mkat/L

- **Markers of cholestasis**

1. Alkaline phosphatase:

[alkaline optimum]; ALP). Half-life= 10 days

Clinical Significance

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

1. 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.

2. Bone ailment: rickets and osteomalacia

3. Liver disease:

4. 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. 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 transfere of the γ–glutamyl group from peptides and compounds that contain it to an acceptor Gammaglutamyl transferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

Clinical Significance

Normal values for GGT	Male: <55 U/L = <0.94 μkat/L
	Female: <38 U/L = <0.65 μkat/L

Causes of raised plasma GGT activity

- Induction of enzyme synthesis, without cell damage, by drugs or alcohol.
- Hepatocellular damage, such as that due to infectious hepatitis:

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.

Normal values for CHE: 4.9-11.9 U/mL
Measurements of CHE activity in serum are used:

1. as a test of liver function

2. as an indicator of possible insecticide poisoning

Causes of decreased plasma cholinesterase activity

1. Hepatic parenchymal disease (reduced synthesis)

2. Ingestion or absorption through the skin, of such anticholinesterases as organophosphates.

Causes of increased plasma cholinesterase activity

1. Recovery from liver damage (actively growing hepatocytes)

2. Nephrotic syndrome

2. Glutamate dehydrogenase

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

Clinical significance

GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease, particularly when interpreted in conjunction with other enzyme test results. The key to this differential diagnostic potential is to be found in the intraorgan and intracellular distribution of the enzyme. As an exclusively mitochondrial enzyme, GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage. GLD activity in serum is stable at 4ºC for 48 hours and at -20ºC for several weeks. The GLD upper reference limits are 6U/L (women) and 8U/ L (men), when a method optimized at 37ºC is used.

C: Muscle enzymes

1-Creatine Kinase(CK)

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 creatine kinase. The concentration gradient is logarithmic

Clinical significance

Normal range for total CK:

Male : 46-171 U/L= 0.78-2.90 μkat/L

Female: 34-145 U/L= 0.58-2.47 μkat/L

Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy (particularly Duchenne sex-linked muscular dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent. Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%- 80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to six-fold increase of CK activity. Quite high values of Ck are noted in viral myositis, polymyositis and similar muscle disease. However in neurogenic muscle disease, such as:

(a) Myasthenia gravis

(b) Multiple sclerosis

(c) Polimyeltis

(d) Parkinsonism

Serum enzyme activity is normal CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant

isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.

CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than five per cent in skeletal muscle: its plasma activity is always high after myocardial infarction. It may be detectable in the plasma of patients with a variety of other disorders in whom the total CK activity is raised, but this accounts for less than six per cent of the total. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Although they have also been reported after brain damage and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease plasma total CK activity is usually normal. Approximate concentrations of tissue CK activity (expressed as multiple activity concentrations in serum and cytoplasmic isoenzyme composition.

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.

LD has a molecular weight of 134 kDa and is composed of four peptide chains of two types: M and H. Each under separate genetic control

The subunit compositions of the five isoenzymes are listed below in order of their decreasing anodal mobility in an alkaline medium.

LD-1 (HHHH; H4)

LD-2 (HHHM; H3M)

LD-3 (HHMM; H2M2)

LD-4 (HMMM; HM3)

LD-5 (MMMM; M4)

Clinical significance

Normal range of total LDH: 180-360 U/L= 3.1-6.1 μkat/L

It is increased in plasma in Myocardial injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more useful in clinching diagnosis between hepatic disease and Myocardial.Injury.

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.
- 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. Extra lysosomal ACPs are also present in many cells:

(a) prostate, (b) bone (osteoclasts), (c) spleen (d) platelets (e) erythrocytes.

The lysosomal and prostatic enzymes are strongly inhibited by d-tartrate ions (tartrate-labile ACP), whereas the erythrocyte and bone isoenzymes are not (TRACP)

Normal range of TR-ACP: 1.5-4.5 U/L= 0.03-0.08 ì kat/L Elevated TR-ACP

(a) Paget disease

(b) Hyperparathyroidism with skeletal involvement

(c) Presence of malignant invasion of bones by cancers

The only nonbone condition in which elevated activities of TR-ACP are found in serum is Gaucher disease of the spleen, a lysosome storage disease.

The main indications for estimation are to help diagnose prostatic carcinoma and to monitor its treatment. The estimation is gradually being replaced by the measurement of plasma prostate specific antigen (PSA) a protein derived from the prostate. This test is more specific and sensitive for diagnosis and monitoring treatment. However, it may be raised in similar circumstances to those affecting prostatic ACP and is more expensive to estimate. ACP is more useful for monitoring the treatment of a known case of disseminated prostatic carcinoma than for making the diagnosis.

2-Glucose -6-phosphate Dehydrogenase

NADP+ oxidoreductase; G6PD) 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. G6PD deficiency

is the most common ezymeopaty , affecting 400 million people worldwide. More than 400 different types of G6PD variants have been described, leading to different enzyme activities associated with a wide range of biochemical and clinical phenotypes.

The majority of G6PD – deficient individuals develop hemolysis only when oxidative stress occurs, as with infections and after ingestion of certain drugs or fava beans. Outside these periods, they are usually asymptomatic; however, G6PD deficiency also leads to mild to sever chronic hemolysis, exacerbated by oxidative stress.

The reference interval for G6PD on erythrocytes is 8-14U/g Hb. Values >18 U/g Hb are often encountered in any condition associated with younger than normal RBCs but are of no clinical significance.