Clinical Enzymology

❖INTRODUCTION

- Enzymes are protein catalysts that increase the rate or velocity of physiologic reactions in our body
- 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
- clinical enzymology refers to measurement of enzyme activity for the diagnosis and treatment of diseases.

Types Of Plasma Enzymes

A. Enzymes that have definite physiological functions in the circulation such as

- Lipoprotein lipase
- Proenzymes of blood coagulation

B. Enzymes that reflect organ pathophysiology

- Normally present in extremely low concentrations in plasma as compared with their high concentrations in various tissues.
- In case of tissue damage, they are released in greater amounts causing higher plasma levels which have diagnostic and sometimes prognostic significance.
- Include:
  - Enzymes present in exocrine secretions, which diffuse passively into the circulation e.g. amylase and lipase in pancreatic disorders.
  - Intracellular enzymes: enzymes used to detect pathological disorders of the various organs of the body e.g. myocardial infarction and hepatitis.

Types Of Plasma Enzymes

	Functional plasma enzymes	Non-functional plasma enzymes
Concentration in plasma	Present in plasma in higher concentrations in comparison to tissues	Normally, present in plasma in very low concentrations in comparison to tissues
Function	Have known functions	No known functions
The substrates	Their substrates are always present in the blood	Their substrates are absent from the blood
Site of synthesis	Liver	Different organs e.g. liver, heart, brain and skeletal muscles
Effect of diseases	Decrease in liver diseases	Different enzymes increase in different organ diseases
Examples	Clotting factors e.g. prothrombin, Lipoprotein lipase and pseudo-choline esterase	ALT, AST, CK, LDH, alkaline phosphatase, acid phosphatase and amylase

Sources of non-functional plasma enzymes

1) **Increase in the rate of enzyme synthesis** e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver diseases.
2) **Obstruction of normal pathway** e.g. obstruction of bile ducts increases alkaline phosphatase.
3) **Increased permeability** of cell membrane as in tissue hypoxia.
4) **Cell damage** with the release of its content of enzymes into the blood e.g. myocardial infarction and viral hepatitis.

Medical importance of non-functional plasma enzymes

- Measurement of non-functional plasma enzymes for:

1. **Diagnosis of diseases**: diseases of different organs cause elevation of different plasma enzymes.

2. **Prognosis of the disease**: follow up the effect of treatment by measuring plasma enzymes before and after treatment.

Assessment of Cell Damage and Proliferation

- Plasma enzyme levels depend on the extent of cell damage and the rate of release from damaged cells.
- 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.

The diagnostic precision of plasma enzyme analysis

1. Estimation of more than one enzyme:

- Many enzymes are widely distributed, but their relative concentrations may vary in different tissues.
- For example, although both ALT and AST are available in the liver, the concentration of AST (GOT) is much greater than that of ALT (GPT) in heart muscle

2. 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.

3. Isoenzyme determination:

- Some enzymes exist in more than one form; these isoenzymes originate in different tissues.
- For example, CK may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium

Factors Affecting Results Of Plasma Enzyme Assays

❑Analytical factors:

1. substrate concentration
2. product concentration
3. enzyme concentration
4. reaction temperature
5. reaction pH
6. presence of activators or inhibitors

Factors Affecting Results Of Plasma Enzyme Assays

❑Non-disease factors

1. **Age:** Plasma AST activity is moderately higher during the neonatal period than in adults. Plasma ALP activity of bony origin is higher in children than in adults.

2. **Sex** : Plasma GGT activity is higher in men than in women. Plasma CK activity is also higher in males.

3. **Race/ethnicity:** Plasma CK activity is higher in black people than in white people.
4. **Physiological conditions:** Plasma ALP activity rises during the last trimester of pregnancy, Why?

5. Several enzymes, such as AST and CK, rise moderately in plasma during and immediately after labour or strenuous exercise.

Measurement of enzyme activity

- **Enzyme activity is expressed in International unit (IU/L)**
- It corresponds to the amount of enzymes that catalyzes the conversion of one micromole of substrate (μmol) to product per minute per liter.

Diagnostic Enzymes In Different Diseases

- Enzyme estimations are helpful in the diagnosis of
  1) Liver diseases
  2) Myocardial Infarction
  3) Muscle diseases
  4) Bone diseases
  5) Cancers
  6) GI Tract diseases

1. **Liver enzymes**
   - **A. Markers of hepatocellulardamage.**
   - **B. Markers of cholestasis**
2. **Pancreatic enzymes**
   - **A. Amylase**
   - **B. Lipase**
   - **C. Trypsin**
3. **Muscle enzymes**
   - **A. Creatine Kinase**
   - **B. Lactate Dehydrogenase**
   - **C. Acid Phosphatase**
   - **D. Glucose -6-phosphate Dehydrogenase**

Detection of Enzymes for diagnosis of disease

Disorders	Enzymes
Liver Function Tests	SGOT, SGPT, ALP, GGT
Cardiac Function Tests	CK-MB, Troponins
Pancreatic Enzymes	Amylase, Lipase
Muscle Enzymes	CK, LDH
Bone Enzymes	ALP , ACP

1.Enzymes For Diagnosis Liver diseases

1. **Markers of hepatocellular damage.:**

- Enzymes which are normally present inside the hepatocytes released into the blood when there is a hepatocellular damage
  - **Alanine aminotransferase (ALT)**
  - **Aspartate aminotransferase (AST)**

2. **Markers of cholestasis :**

- Enzymes which are primary membrane bound (plasma membrane or side of hepatocytes)
  - **Alkaline phosphatase,**
  - **Gamma glutamyl transferase (GGT)**
  - **5-nucleotidase**

1. Markers of hepatocellular damage

❑ Aminotransferases/Transaminases

- The transaminases are enzymes involved in the transfer of an amino group from **a 2-amino acid to a 2- keto acid**
- The aminotransferases are used as part of the **biochemical liver profile**.
- **Aminotransferases** are **sensitive indicators of liver cell injury** and are most helpful in recognizing acute hepatocellular diseases such as hepatitis
- These enzymes are released into the blood in greater amounts when there is damage to the liver cell membrane resulting in increased permeability.

❑ **Types of plasma Aminotransferases**

- **Aspartate transaminase (AST)** enzyme, it is also called serum glutamic oxalacetic transaminase(SGOT)
- **Alanine transaminase (ALT)** enzyme, it is also called serum glutamic pyruvic transaminase (SGPT)

❑ Aminotransferases/Transaminases

- **AST and ALT enzymes** are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.
- **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
- According to AST/ALT ratio, we can estimate the extent of the liver damage.
- Elevated **plasma ALT** are considered to be relatively **specific for liver disease than AST** as AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.
- Aminotransferases levels **> 1000 IU/L** occur almost exclusively in disorders associated with extensive hepatocellular injury

Clinical Significance

- **Normal values of AST:**
  - Male: <35 U/L Female: <31 U/L
- **Normal values of ALT:**
  - Male: <45 U/ Female: <34 U/L

2.Markers of cholestasis

A. Alkaline phosphatase (ALP)

- **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 placent**.
- Normal level-0-45 IU/L
- In adult, 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:

- **During pregnancy**: the plasma total ALP activity rises due to the contribution of the placental isoenzyme.
- **In infants** : plasma total ALP activity is up to five times than in adults, and consists predominantly of the bone isoenzyme.
- **In children**: the total activity increases by about 2 to 5 times during the pubertal bone growth

2. Pathological

❑ **Liver disease**

- Intrahepatic or extrahepatic cholestasis
- Inflammatory bowel disease: the gut ALP isoenzyme can be increased in ulcerative colitis.

❑ **Bone disease :**

- Rickets (in child) and osteomalacia.
- Paget's disease of bone (may be very high)
- osteogenic sarcoma
- hyperparathyroidism.

❑ **Malignancy:** bone or liver involvement or direct tumour production

Isozymes Of Alkaline Phosphatase (ALP)

1) **Hepatic Isoenzyme:** Its level rises in extra hepatic biliary obstruction.
2) **Bone Isoenzyme:** Increases due to osteoblastic activity and is normally elevated in children during periods of active growth
3) **Placental Isoenzyme :** Rises during last 6 weeks of pregnancy.
4) **Intestinal Isoenzyme:** Rise occurs after a fatty meal. May increase during various GI disorders.

2.Markers of cholestasis

B. Gamma-glutamyl-transferase

- **Gamma-glutamyl-transferase** catalyzes the transfere of the γ–glutamyl group from peptides and compounds that contain it to an acceptor.
- It is involved in **amino acid transport** across the membranes.
- **Gamma-glutamyl-transferase** is found mainly in **biliary ducts of the liver**, kidney and pancreas.
- Y-GGT increased in liver diseases especially in obstructive jaundice.

➢ Clinical Significance

Normal values for GGT

- Male: <55 U/L Female: <38 U/L

❑ Causes of raised plasma GGT activity

1) Induction of enzyme synthesis, without cell damage, by drugs or alcohol.
2) Hepatocellular damage, such as that due to infectious hepatitis
3) Cholestatic liver disease, elevated GGT activity usually parallel those of ALP activity

2.Markers Of Cholestasis

C. 5’ Nucleotidase

- The enzyme hydrolyses 5’ nucleotides to 5’ nucleosides at an optimum pH of 7.5
- Moderately increased in **hepatitis and highly elevated in biliary obstruction**.
- Unlike ALP the level is unrelated to osteoblastic activity and is thus **unaffected by bone disease.**

2.Enzymes For Diagnosis Pancreatic Diseases

1. Amylase

- It 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.
- **Plasma amylase** is derived from the **pancreas and salivary glands**. Thus, pancreatic juice and saliva contain high concentration of amylase
- Estimation of plasma amylase activity is mainly use to diagnosis of acute pancreatitis
- **Clinical Significance**
  - Normal values of amylase: 28-100 U/L

Causes of Raised Plasma Amylase Activity

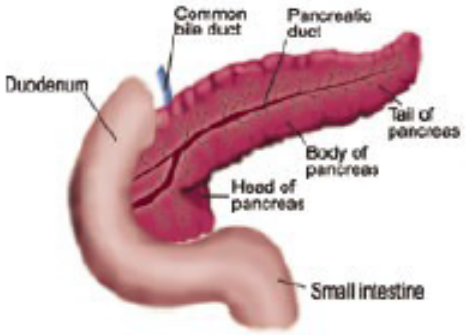
1) **Marked increase (5 to 10 times the upper reference limit)**:
   - Acute pancreatitis
   - Severe glomerular impairment
2) **Moderate increase (up to five times the upper reference limit)**
   - **Acute abdominal disorders**
     - Acute cholecystitis((inflammation of the gall bladder)
     - Perforated peptic ulcer
     - Intestinal obstruction
     - **Salivary gland disorders** like mumps, salivary calculi

2. Lipase

- **Lipase is** a single chain glycoprotein with molecular weight of 48 kDa.
- Lipase is derived from the pancreas but is more specific for pancreatic pathology than amylase.
- In addition, lipase has a longer half-life than amylase , therefore may be more useful in the diagnosis of 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
- **Clinical Significance**

• Normal values: 40-200 U/L

Pancreatic Enzymes



AMYLASE	LIPASE
N: 25-125 U/L	N: 10-150 U/L
↑acute pancreatitis	↑acute pancreatitis
↑ starts at 4-12 h	↑ starts at 12-18 h
maximum 12-24 h	
Returns N 48-72 h	Returns N 5-7 days

3. Trypsin

- **Trypsin** is a serine 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

3. Plasma Enzymes For Diagnosis skeletal Muscle Diseases

1. Total creatine kinase (CK)

- CK-MM: marked increase
- CK-MB: less marked increase

2. Lactate dehydrogenase LDH-5

Plasma Enzymes For Diagnosis Muscle Diseases

A. Creatine Kinase (CK)

- **Creatine phosphokinase (CPK)** It is an enzyme found primarily in the heart and skeletal muscles, and to a lesser extent in the brain but not found at all in liver and kidney.

- Catalyzes the transfer of phosphate between creatine and ATP/ADP Provides rapid regeneration of ATP when ATP is low

- In tissues and cells that consume ATP rapidly, especially skeletal muscle, **CPK** serves as an energy reservoir for the rapid buffering and regeneration of ATP *in situ*

- Creatine + ATP ⟶ phosphocreatine + ADP

- **Clinical significance**

- Normal range for total CK:
  - Male : 46-171 U/L
  - Female: 34-145 U/L

Causes of raised plasma creatine kinase (CK) activities

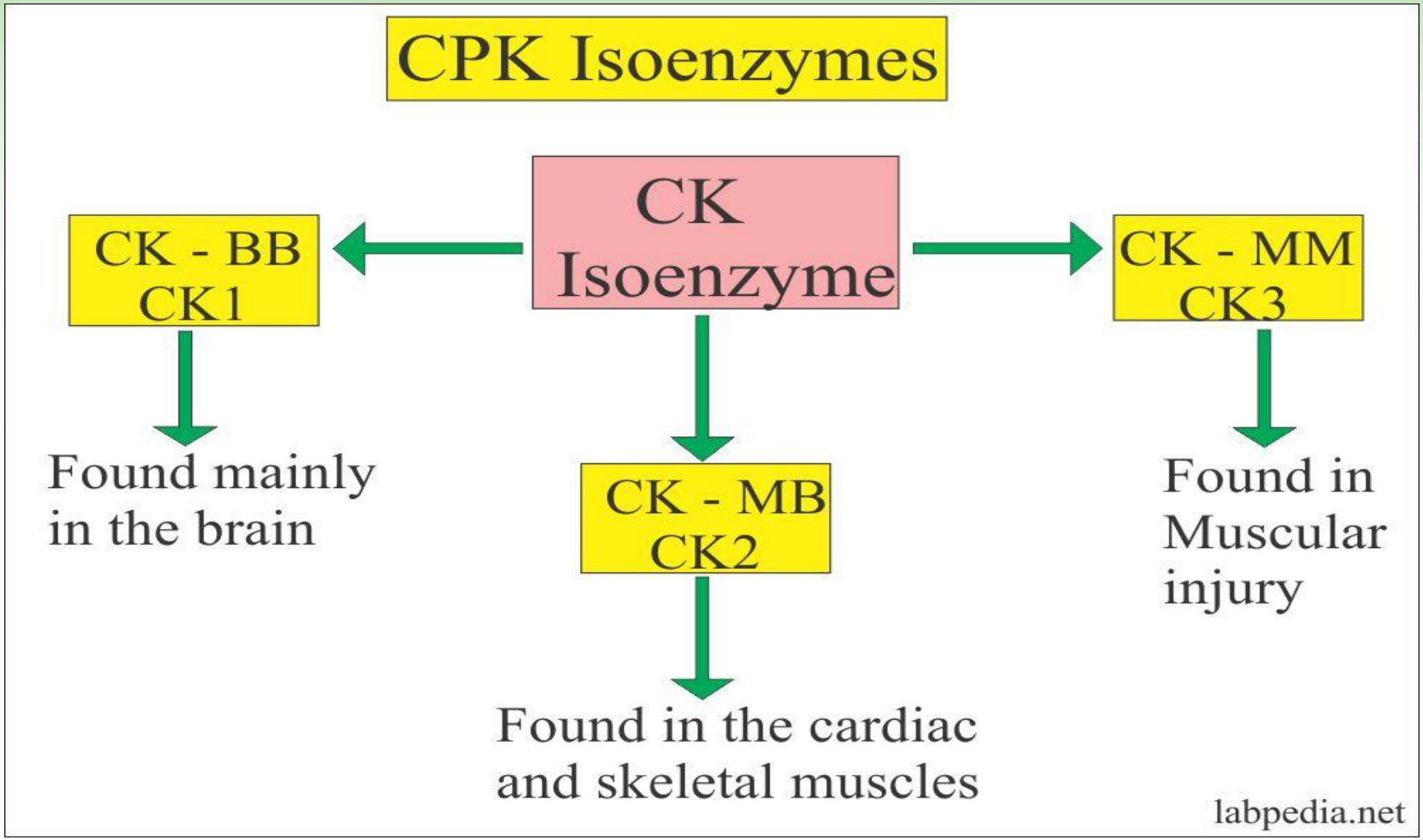
1. **Physiological :** enzyme activity in serum is highest in infancy and childhood (7-10 years of age)
2. **pathological :**
   - Clinical significance Elevation of CK is an indication of damage to muscle.
   - Clinically, creatine kinase is assayed in blood tests as a marker of damage of CK-rich tissue such as in
     A. myocardial infarction (heart attack)
     B. rhabdomyolysis (severe muscle breakdown)
     C. muscular dystrophy
     D. autoimmune myositides
     E. acute kidney injury

Isoenzymes of Creatine Kinase CK

- **Creatine Kinase activity is found greatest in skeletal muscles, brain and heart tissue.**
- **Isoenzymes of CK: CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes**
- **Three isoenzymes:**

- **CK1 (CK-BB):** in the brain
- **CK2 (CK-MB**): mostly in the cardiac muscles
- **CK3(CK-MM):** in both the skeletal and the cardiac muscles.

- **CK-MB**
  - **is more specific for cardiac muscle, so it is a more sensitive marker of myocardial injury than total CK activity.**
  - **It accounts for about 35% of the total CK activity in cardiac muscle and less than 5% in skeletal muscle: its plasma activity is always high after myocardial infarction.**
- **CK-BB**
  - **high concentrations in the brain and after brain damage**
  - **CK-BB increased plasma activities may occur during parturition (childbirth).**


CPK Isoenzymes
CK Isoenzyme
CK - BB CK1
CK - MB CK2
CK - MM CK3
Found mainly in the brain
Found in the cardiac and skeletal muscles
Found in Muscular injury
labpedia.net

B. Lactate Dehydrogenase(LDH)

- **LDH (Lactate dehydrogenase)** is an enzyme that produces energy.
- It Catalyses the reversible interconversion of lactate and pyruvate.
- A dehydrogenase is an enzyme that transfers a hydride from one molecule to another.
- LDH is extensively found in body tissues with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes
- Measurement of plasma total LDH activity is therefore a non-specific marker of cell damage
- LDH released during tissue damage, common marker of injuries and diseases such as heart failure.
- **Clinical significance:**
  - Normal Values Of LDH: 140u/l to 280 u/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.

Lactate Dehydrogenase Isoenzymes

- LDH enzyme is tetramer with 4 subunits.
- The subunit may be either H(Heart) or M(Muscle) polypeptide chains.

- The subunit compositions of the five isoenzymes :
  I. **LD1 (H4),** found in heart and in RBC as well as kidney.
  II. **LD2 (H3M),** found in the reticuloendothelial system.
  III. **LD3 (H2M2),** found in the lungs.
  IV. **LD4 (HM3)** found in the kidneys, placenta and pancrease.
  V. **LD5 (M4).**found in the liver and skeletal muscle

- Elevation of LD1 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.

- 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

Causes of Raised Plasma Total LDH Activity

1. **Artefactual** *:* In vitro haemolysis or delayed separation of plasma from whole bloo
2. **Marked increase (may be greater than 5–10 times the URL)**:
   - Circulatory failure with ‘shock’ and hypoxia
   - Myocardial infarction.
   - In some haematological disorders: megaloblastic anaemia, acute leukaemias and lymphomas
3. **Moderate increase**.(usually less than five times)
   - viral hepatitis
   - malignancy of any tissue
   - skeletal muscle disease
   - pulmonary embolism: **Pulmonary embolism** is the sudden blockage of a major blood vessel (artery) in the **lung**

4. Plasma Enzymes For Diagnosis Of Myocardial Infarction

- Enzyme assays for the diagnosis of Acute Myocardial Infarction are:

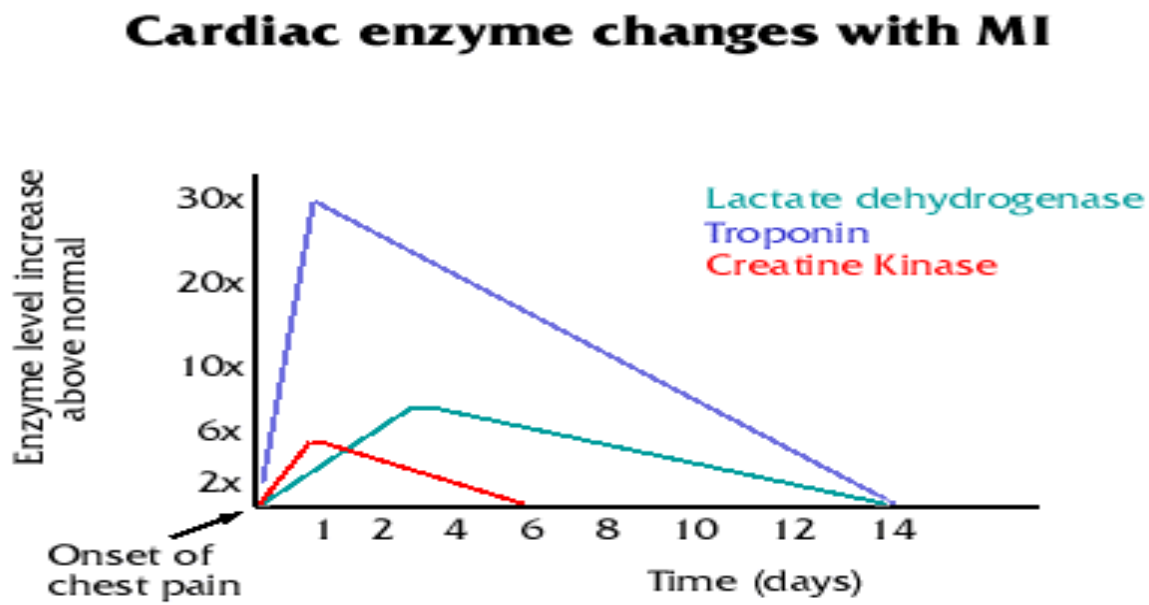
1. **Creatine kinase (CK).**
2. **Creatine kinase-MB (CK-MB) isoenzyme**
3. **Lactate dehydrogenase (LD).**
4. **Aspartate aminotransferase (AST).**

Plasma Enzymes For Diagnosis Of Myocardial Infarction

1. Creatine Phosphokinase (CK/ CPK)

- Phosphocreatine serves as energy reserve during muscle contraction
- Mainly useful in detecting damage to myocardial and skeletal muscle tissue
- It is composed of two polypeptide chains designated M (muscle) and B (brain)
- Three isoenzymes are present human tissues.
  - CK-BB (Brain form) N: Absent in blood
  - CK-MB (Cardiac form) N < 6% of total CK activity
  - CK-MM (Skeletal Ms. form) N > 95% of total activity
- Reference range of total CK activity:
  - Males: Up to 180 U/L Females: 25-40%lower than in males
- ↑ total CK activity
  - Myocardial damage
  - Skeletal muscle damage.
  - Muscular exercise
  - Drugs e.g. digoxin and lidocaine
- ↑ CK- MB isoenzyme : myocardial damage

- **Time sequence of serum total CK after myocardial infarction**
  - **begins to rise 4-6 hours**
  - **peaks 24 hours**
  - **returns to normal in 3-4 days**
- **Time sequence of CK-MB**
  - **Rises and returns to normal sooner than total CK**
  - **Rises in 3-4 hours**
  - **Returns to normal in 2 days**



2. Aspartate Amino Transferase (AST):-

- **The level is significantly elevated in Acute MI.**
- **Normal Value:- 0-41 IU/L at 37°C**
- **In acute MI- Serum activity rises sharply within the first 12 hours, with a peak level at 24 hours or over and returns to normal within 3-5 days.**
- **The rise depends on the extent of infarction**
  - **> 350 IU/L are due to massive infarction (Fatal)**

3.Lactate Dehydrogenase (LDH):-

- **Present in almost all tissues**
- **LDH** catalyzes the reversible conversion of pyruvate and lactate.
- **Most often measured in myocardial infraction.**
- **Normal level :** 55-140 IU/L at 30°C.
- **Time sequence of LD after myocardial infarction**
  - **↑ after 12-24 hours**
  - **peak after 3-5 days**
  - **return to normal after 7-12 days**
- **The magnitude of rise is proportional to the extent of myocardial infarction.**

5.Enzymes For Diagnosis Of Prostate Diseases

❑Acid Phosphatase(ACP) :Include all phosphatases that hydrolyze phosphate esters with an optimum pH of less than 7.0.

- ❑ It is produced by Primarily in prostate gland. It is also found in erythrocytes, platelets, leukocytes, bone marrow, bone, liver, spleen, kidney, and intestine.
- ❑ Normal range of ACP: 1.5-4.5 U/L

❑Clinical Implications:

- Primarily used to diagnose prostate cancer and to monitor its treatment
- ACP is 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
- In other non prostatic conditions e.g.
  - A. Hemolysis
  - B. Paget disease
  - C. Hyperparathyroidism with skeletal involvement
  - D. Presence of malignant invasion of bones by cancers

6.Enzymes For Diagnosis Of Bone Diseases

❑Alkaline Phosphatase(ALP):-

1. Rises in Rickets, osteomalacia,
2. hyperparathyroidism and in Paget's disease.
3. Also rises in primaryand secondary malignancies of bones.

❑ Acid Phosphatase(ACP):-

- Highly increased in bony metastasis of carcinoma prostate

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

CONCLUSION

- Enzymes are biological catalyst present in every cell of the body.
- Important enzymes in investigation of liver diseases are AST ,ALT ,ALP , and y-GT.
- Important enzymes in investigation of heart diseases are CK ,LDH, and AST.
- ALP can be used in the investigation of liver and bone diseases.