Enzymes in Medicine

Diagnostic significance of enzymes

1- Enzymes can act as diagnostic markers of underlying diseases and their management.

2- Enzymes can also act as reagents for various biochemical estimations and detections

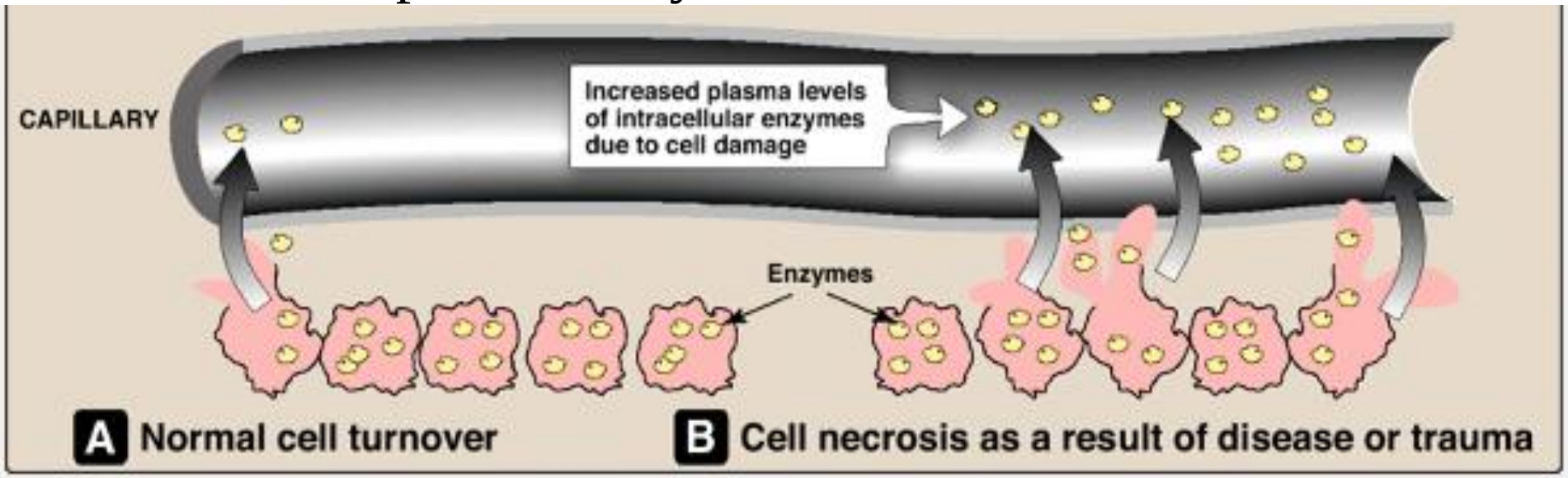
Enzymes as diagnostic markers

1- Functional plasma enzymes (Plasma derived enzymes):

- Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood.
- Examples of these functional plasma enzymes include: lipoprotein lipase, pseudo cholinesterase, and the proenzymes of blood coagulation and blood clot dissolution
- Most of these enzymes are synthesized in and secreted by liver.

2- Nonfunctional plasma enzymes (Cell derived enzymes):

- Plasma also contains numerous other enzymes that perform no known physiologic function in blood.
- These apparently nonfunctional plasma enzymes arise from the routine normal destruction of erythrocytes, leukocytes, and other cells.
- Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes.



Possible mechanisms responsible for:

A- increased serum levels

1- Increased release

- Necrosis of cell
- Increased permeability of cell without gross cellular damage
- Increased production of enzyme within the cell resulting in increase in serum by overflow
- Increase in tissue source of enzyme as in malignancy

2- Impaired disposition

- Increased levels in obstructive jaundice
- Increased levels in renal failure

B- Decreased serum levels

- Decreased formation which may be Genetic or Acquired
- Enzyme inhibition
- Lack of cofactors

Clinical significance of enzyme estimation

- Single or serial assay of serum activity of a selected enzyme
  1- Helps in making the diagnosis/differential diagnosis/ early detection of a disease
  2- Helps in ascertaining prognosis of a disease
  3- Helps in ascertaining the response to drugs in a disease
  4- Also help in ascertaining the time course of disease.

Enzymes as diagnostic markers in different diseases

Enzyme estimations are helpful in the diagnosis of :

1- Myocardial Infarction
2- Liver diseases
3- Muscle diseases
4- Bone diseases
5- Cancers
6- GI Tract diseases

Diagnosis of AMI

-The diagnosis of AMI is usually predicated on the WHO criteria of chest pain, ECG changes, and increases in biochemical markers of myocardial injury.

- Half of the patients with "typical" symptoms do not have AMI.

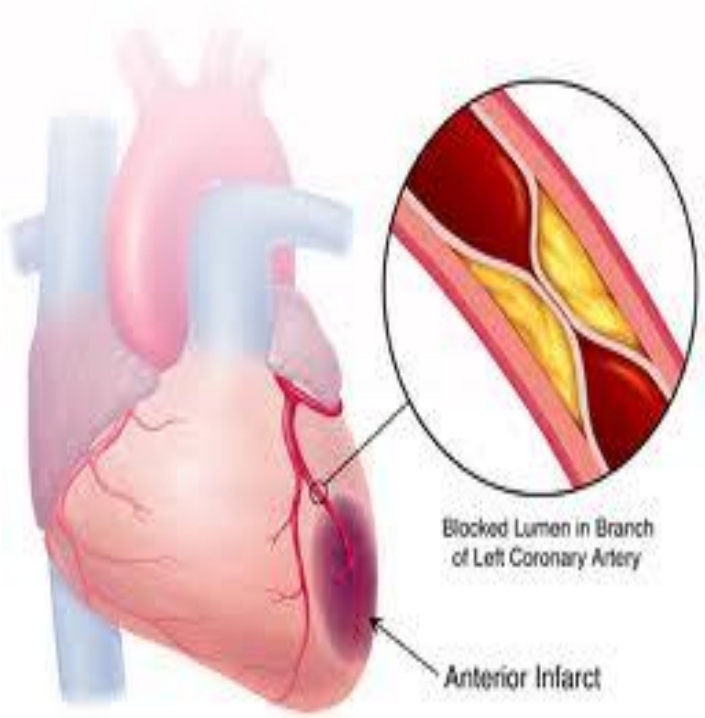
- The ECG is specific for AMI, but lacks sensitivity.

- In contrast, biochemical markers have excellent sensitivity for diagnosing AMI.

- By combining the most sensitive and the most specific tests, diagnostic accuracy can be enhanced.

Serum enzymes and other biomarkers in acute MI

- Enzyme assays routinely carried out for the diagnosis of acute myocardial infarction are:
- SGOT (AST)
- LDH - CPK
- CPK isoforms by electrophoresis
- CPK - MB by immunoinhibition
- Myoglobin
- CPK- MB by mass immunoassay
- Troponin T - Troponin I



- Brain natriuretic peptide (BNP), a reliable marker of ventricular function.
- Acceptable biochemical markers of ischemic heart disease are now considered to include myoglobin, CK-MB, total CK, and cardiac troponins T and I.

- Of these, troponins and CK-MB are the sensitive and specific markers, whereas myoglobin though sensitive, is non-specific.

1- <u>Creatine kinase (CPK)</u>

- 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
- After myocardial infarction- serum value is found to increase within 3-6 hours, reaches a peak level in 24- 30 hours and returns to normal level in 2-4 days (usually in 72 hours).
- Normal Value: serum activity varies from 30 to 145 U/L in females and 55 to 170 U/L in males.
- CK is a sensitive indicator in the early stages of myocardial ischemia.
- No increase in activity in heart failure and coronary insufficiency.
- In acute MI, CPK usually rises faster than AST and returns to normal faster than the AST.

CK/CPK isoenzymes

- There are three Isoenzymes (M.W. 80 kDa).
- Measuring them is of value in the presence of elevated levels of CK or CPK to determine the source of the elevation.
- Each isoenzyme is a dimer composed of two protomers 'M' (for muscles) and 'B'(for Brain).
- These isoenzymes can be separated by, electrophoresis.

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean % in blood
MM(CK3)	Least	Skeletal muscle Heart muscle	97-100%
MB(CK2)	Intermediate	Heart muscle	0-3%
BB(CK1)	Maximum	Brain	0%

- Normal levels of CPK are almost entirely MM, from skeletal muscle.
- Elevated levels of CPK resulting from acute myocardial infarction are about half MM and half MB.

- Myocardial muscle is the only tissue that contains more than five percent of the total CK activity as the CK2 isoenzyme, in the patients with recent myocardial infarction, the total isoenzyme is elevated up to 20-folds above the normal.

2- <u>Aspartate amino transferase (AST)</u>

- It is called as serum SGOT or AST.
- The level is significantly elevated in Acute MI.
- Normal Value: 8-33 U/L.

- 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.
- Re- infarction results in secondary rise of AST.
- Prognostic significance:

Levels > 350 U/L are due to massive infarction (Fatal)
Levels > 150 U/L are associated with high mortality
Levels < 50 U/L are associated with low mortality.

Other diseases:

- The rise in activity is also observed in muscle and hepatic diseases.
- These can be well differentiated from simultaneous estimations of other enzyme activities like ALT etc, which do not show and rise in activity in Acute MI.

3- Lactate dehydrogenase (LDH)

- It catalyzes the reversible conversion of pyruvate and lactate.
- Normal level: 140-280 U/L.
- The levels in the upper range are generally seen in children.
- LDH level is 100 times more inside the RBCs than in plasma, and therefore minor amount of hemolysis results in false positive result.
- In Acute MI: The serum activity rises within 12 to 24 hours, attains a peak at 48 hours (2 to 4 days) reaching about 1000 U/L and then returns gradually to normal from 8 - 14 day.
- The magnitude of rise is proportional to the extent of myocardial infarction.
- Serum LDH elevation may persist for more than a week after CPK and SGOT levels have returned to normal levels.

Other diseases:

-The increase in serum activity of LDH is also seen in hemolytic anemias, hepatocellular damage, muscular dystrophies, carcinoma, leukemia, and any condition which causes necrosis of the body cells.

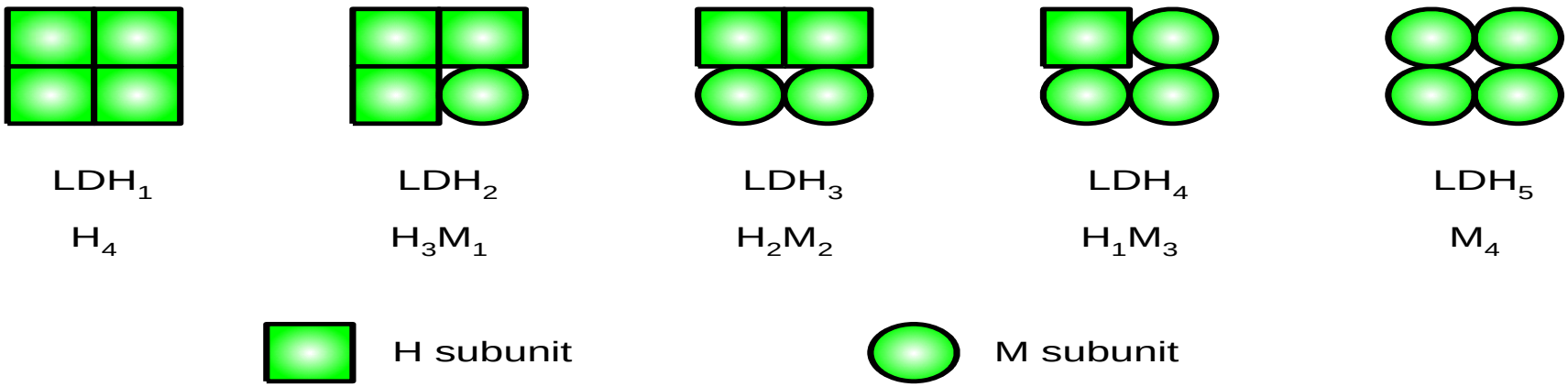
- Since the total LDH is increased in many diseases, so the study of isoenzymes of LDH is of more significance.

Isoenzymes of LDH

- LDH enzyme is tetramer with 4 subunits.
- The subunit may be either H (Heart) or M (Muscle) polypeptide chains.
- These two chains are the product of 2 different genes.
- Although both of them have the same molecular weight, there are minor amino acid variations.

- There can be 5 possible combinations; H4, H3M1, H2M2, H1M3. M4 (5 different isoenzymes in all individuals).

Isoenzymes of LDH



No. of Isoenzyme	Subunits of isoenzyme	Electrophoretic mobility (pH 8.6)	Tissue origin	human serum%
LDH-1	H4	Fastest	Heart muscle	14-26%
LDH-2	H3M1	Faster	RBCs	29-39%
LDH-3	H2M2	Fast	Brain	20-26%
LDH-4	H1M3	Slow	Liver	8-16%
LDH-5	M4	Slowest	Skeletal Muscles	6-16%

4- Cardiac troponins

-They are not enzymes, but accepted as markers of MI.

- The Troponin complex consists of 3 components; Troponin C (calcium binding), Troponin I (actinomyosin ATPase inhibitory element), and Troponin T (tropomyosin binding element).
- Troponin I is released in to the circulation within 3-6 hours of cardiac manifestations onset, peak is observed at 1-24 hours and remains elevated for 4-7 days post infarction.
- Serum level of TnT increases within 4-6 hours of myocardial infarction, peaks at 10-24 hours and then remains elevated up to 10-24 days.

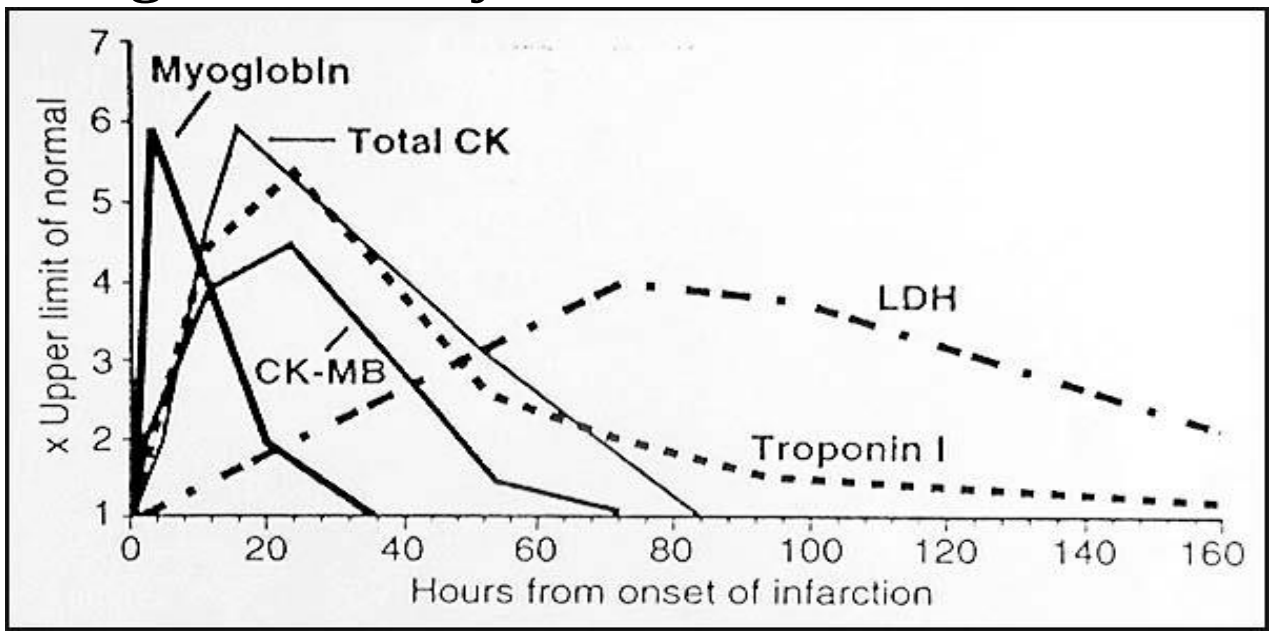
-The TnT2 estimation is 100% sensitive index for myocardial infarction.

Brain Natriuretic Peptide (BNP)

- The natriuretic peptide family consists of three peptides:
- Atrial natriuretic peptide (ANP),
- Brain natriuretic peptide (BNP), and
- C-type natriuretic peptide (CNP), its clinical significance of is not clear.
- ANP is produced primarily in the cardiac atria.
- BNP is present in human brain, but more in the cardiac ventricles.
- Greatest proportion of circulating BNP is thought to come from the ventricles (left).
- Therefore it is a reliable marker of ventricular function
- Patients with congestive heart failure have high plasma concentrations of ANP and BNP.

5- Myoglobin as cardiac marker

- One of earliest markers is myoglobin, which is very sensitive but, in certain clinical settings, lacks specificity.
- Its level rises within 1 hour of infarction from damaged tissues.
- Falsely high levels may be observed in patients of renal failure or patients having muscle injuries.



Enzymatic activity changes in acute MI

Serum enzymes in liver diseases

- Serum enzyme tests can be grouped into two categories:

1- enzymes whose elevation in serum reflects damage to hepatocytes

2- enzymes whose elevation in serum reflects cholestasis.

1- Enzymes that reflect damage to hepatocytes

- The aminotransferases (transaminases) are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases such as hepatitis.
- These include:
  1- Aspartate aminotransferase (AST).
  2- Alanine aminotransferase (ALT).

Aminotransferases

- AST is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes in decreasing order of concentration.
- Normal level: 8-33 U/L
- ALT is found primarily in the liver, its normal level: 4-36 U/L
- The aminotransferases are normally present in the serum in low concentrations.
- These enzymes are released into the blood in greater amounts when there is damage to the liver cell membrane resulting in increased permeability.

Diagnostic significance of aminotransferases

- Levels of up to 300 U/L are nonspecific and may be found in any type of liver disorder.
- Striking elevations: i.e., aminotransferases > 1000 U/L—occur almost exclusively in disorders associated with extensive hepatocellular injury such as (1) viral hepatitis, (2) ischemic liver injury (prolonged hypotension or acute heart failure), or (3) toxin- or drug-induced liver injury.
- In most acute hepatocellular disorders, the ALT is higher than or equal to the AST.
- An AST:ALT ratio > 2:1 is suggestive while a ratio > 3:1 is highly suggestive of alcoholic liver disease.
- The AST in alcoholic liver disease is rarely >300 U/L and the ALT is often normal.

- A low level of ALT in the serum is due to an alcohol-induced deficiency of Pyridoxal phosphate.
- In obstructive jaundice the aminotransferases are usually not greatly elevated.

2- <u>Enzymes that reflect cholestasis</u>

- The activities of three enzymes:

1- Alkaline phosphatase (ALP)

2- 5'-nucleotidase, and

3- γ-Glutamyl transpeptidase (GGT): are usually elevated in cholestasis.

- ALP and 5'-nucleotidase are found in or near the bile canalicular membrane of hepatocytes, while GGT is located in the endoplasmic reticulum and in bile duct epithelial cells.

ALP in liver diseases

- The normal serum ALP consists of many distinct isoenzymes found in the liver, bone, placenta, and, less commonly, small intestine. Its normal level is 44 to 147 IU/L.

Physiological variations:

- Patients over age 60 can have a mildly elevated ALP.
- Individuals with blood types O and B can have an elevation of the serum ALP
- After eating a fatty meal due to the influx of intestinal ALP into the blood.
- It is also non-pathologically elevated in children and adolescents undergoing rapid bone growth, because of bone ALP.
- It is also high late in normal pregnancy due to the influx of placental ALP.

Pathological variations:

- Elevation of liver-derived ALP is not totally specific for cholestasis, and a less than threefold elevation can be seen in almost any type of liver disease.

- ALP elevations greater than four times normal occur primarily in patients with cholestatic liver disorders, infiltrative liver diseases such as cancer and amyloidosis.

- In liver diseases, the elevation is almost always due to increased amounts of the liver isoenzyme.

- In the absence of jaundice or ↑ aminotransferases, an elevated ALP of liver origin often, but not always, suggests early cholestasis and, less often, hepatic infiltration by tumor.

Intrahepatic cholestasis:

- Values are increased in drug-induced hepatitis, primary biliary cirrhosis, rejection of transplanted liver, and, rarely, alcohol-induced steatonecrosis.

Extrahepatic cholestasis:

- Very high values are found in obstructive jaundice due to cancer, common duct stone, sclerosing cholangitis, or bile duct stricture

- The level of serum ALP elevation is not helpful in distinguishing between intrahepatic and extrahepatic cholestasis.

Isozymes ALP:

1- Hepatic isoenzyme: its level ↑ in extra hepatic biliary obstruction.
2- Bone isoenzyme: ↑ due to osteoblastic activity and is normally ↑ in children during periods of active growth.
3- Placental isoenzyme: ↑ during last 6 weeks of pregnancy.
4- Intestinal isoenzyme- ↑ after a fatty meal and may ↑ during various GI disorders.
5- Regan isoenzyme: present in plasma of about 15% of patients with carcinoma of lung, liver or gut. Also, seen in chronic smokers. structurally resembles placental ALP
6- Nagao isoenzyme: a variant of Regan isoenzyme: detected in metastatic carcinoma of pleural surfaces and adenocarcinoma of pancreas and bile duct.

γ glutamyl transferase (transpeptidase) (GGT)

- It is involved in amino acid transport across the membranes.
- Found mainly in biliary ducts of the liver, kidney and pancreas.
- Enzyme activity is induced by a number of drugs and in particular alcohol.
- Normal serum value of GGT below age of 45 is 5-27 U/L and above the age of 45, it is 8-38 U/L.
- Increased in infective hepatitis and prostate cancers.
- γ-GT is highly increased in liver diseases especially in obstructive jaundice and in liver neoplasms.
- γ-GT levels are used as a marker of alcohol induced liver disease and in liver cirrhosis.

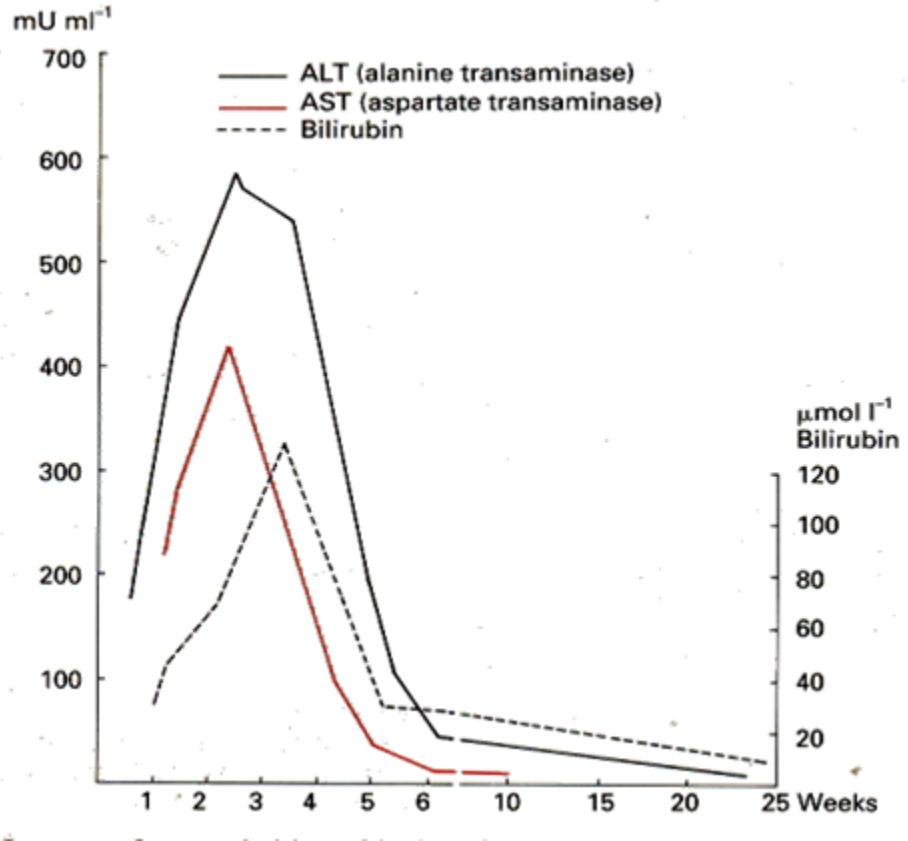
5 ’ nucleotidase (nucleotide phosphatase)

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

Serum enzymes in liver diseases

In viral hepatitis

Rapid rise in transaminases (AST & ALT) in serum occurs even before bilirubin rise is seen



3- Serum enzymes in bone diseases

- ALP: ↑ in Rickets, osteomalacia, hyperparathyroidism and in Paget's disease. Also ↑ in primary and secondary malignancies of bones.
- Acid Phosphatase: Highly ↑ in bony metastasis of carcinoma prostate, it hydrolyses phosphoric acid esters at pH between 4 and 6. Also, present in high concentration in semen, a finding which is used in forensic medicine in investigation of rape.

4- Serum enzymes in muscle disease

- **Aldolase**: moderate increase in dermatomyositis, muscular dystrophies, highest values are seen in Deuchenne type of muscular dystrophies (Normal range of serum is 1.5-7 U/L)
- **CPK**: ↑ in neurogenic muscular dystrophies, highest values are seen in Deuchenne type of muscular dystrophies

5- Serum enzymes in GI tract diseases

Amylase:

- Normal serum value is 40- 140 U/L
- Serum activity > 1000 units is seen within 24 hours in acute pancreatitis, values are diagnostic.
- A raised serum activity is also seen in perforated peptic ulcer and intestinal obstruction.

Lipase:

- Levels as high as 2800 U/l are seen in acute pancreatitis and persists for 7-14 days, it remains elevated longer than amylase, also, increased in mumps
- Also reported high in perforated duodenal and peptic ulcers and intestinal obstruction.
- Normal serum range is 0-160 U/L.

Enolase

- It is a glycolytic enzyme. Neuron-specific enolase (NSE) is an iso-enzyme seen in neural tissues.
- NSE is a tumor marker for cancers associated with neuro – endocrine origin, small cell lung cancer, neuroblastoma, pheochromocytoma, medullary carcinoma of thyroid, etc.
- Upper limit of NSE is 15-30 ng/mL.

Prostate specific antigen (PSA)

- Produced from the secretory epithelium of prostate gland.
- It is normally secreted into seminal fluid
- Normal value is 4 ng/ml. It is very specific for prostate activity.
- Values between 4-10 ng/ml is seen in benign prostate enlargement; but values above 10 ng/ml is indicative of prostate cancer.

Cholinesterase

-This enzyme is secreted by the liver into the blood- stream and low plasma activities occur in chronic hepatic dysfunction.

- Low activities occur physiologically during pregnancy.
- Interest in this enzyme derives largely from the fact that it hydrolyzes a muscle-relaxant drug, widely used in anaesthesia, called succinyl choline (scoline).
- Acetyl cholinesterase (true cholinesterase or Type 1 cholinesterase) can act mainly on acetylcholine.
- Normal serum range is 3.070–8.483 U/L in adult women, 4.687–9.116 U/L in adult males
- It is present in nerve endings and in RBCs.
- Plasma cholinesterase activity also falls in organophosphate poisoning.
- Organophosphorus insecticides (Parathione) irreversibly inhibit cholinesterase in RBCs.

-Measurement of cholinesterase level in RBCs is useful to determine the amount of exposure in persons working with these insecticides.
- Pseudocholinesterase or type II cholinesterase is non-specific and can hydrolyze acyl esters. It is produced mainly by liver cells.
- Normal serum level is 8-18 U/ml.

Glucose-6-phosphate dehydrogenase

- Key enzyme regulatory of PPP.
- Normal value of G6PD 8.6 to 18.6 U/gm of hemoglobin.
- It is mainly used for production of NADPH.
- It has a special role in the RBC metabolism.

6- Enzymes as tumor markers

Enzyme	Disease
Serum acid phosphatase	Cancer prostate
Serum Alkaline phosphatase	Metastasis in liver, jaundice due to carcinoma head of pancreas, osteoblastic metastasis in bones
Serum LDH	Advanced malignancies and Leukemias
β- Glucuronidase	Cancer of urinary bladder
Leucine Amino Peptidase (LAP)	Liver cell carcinoma
Neuron specific Enolase	Malignancies of nervous tissue and brain

Enzymes as therapeutic agents

Enzyme	Therapeutic Application
Streptokinase / Urokinase	Acute MI, Pulmonary embolism, DVT(Deep vein thrombosis)
Trypsin, lipase and amylase	Pancreatic insufficiency
Asparaginase /Glutaminase	Acute lymphoblastic leukemias
Hyaluronidase	Enhanced local anesthesia and for easy diffusion of fluids
Papain	Anti inflammatory
Chymotrypsin	Pain killer and Anti inflammatory
Alpha- 1 Antitrypsin	Deficiency and Emphysema
Serratopeptidase	Pain killer and Anti inflammatory

Summary: enzymes as diagnostic markers

Name of the enzyme	Conditions in which Serum level is elevated
Aspartate Amino transferase (AST)	Myocardial infarction, Liver disease especially with liver cell damage
Alanine Amino transferase (ALT)	Liver disease especially with liver cell damage
Alkaline Phosphatase (ALP)	Liver disease- biliary obstruction Osteoblastic bone disease-rickets
Acid Phosphatase (ACP)	Prostatic carcinoma
γ glutamyl Transferase (γ GT)	Liver disorder like liver cirrhosis and alcoholism
Creatine kinase (CK)	Myocardial infarction and skeletal muscle disease (muscular dystrophy)
Lactate Dehydrogenase (LDH)	Myocardial infarction, other diseases like liver diseases, some blood diseases
α Amylase	Acute pancreatitis