Tishk International University
Faculty of Applied Science
Nutrition and Dietetics Department
Nutritional Biochemistry II
2nd Grade

Clinical Enzymology

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Objectives

- Clinical Enzymology
- Diagnostic significance of enzymes
- Enzymes related to different tissues and organs
- Enzymes as tumor markers

Enzymes in Clinical Diagnosis

Plasma enzymes can be classified into 2 major groups:

1. Small group of enzymes are actively secreted into the blood by certain cell types.

e.g. the liver secretes zymogens(inactive precursors) of the enzymes involved in blood coagulation.

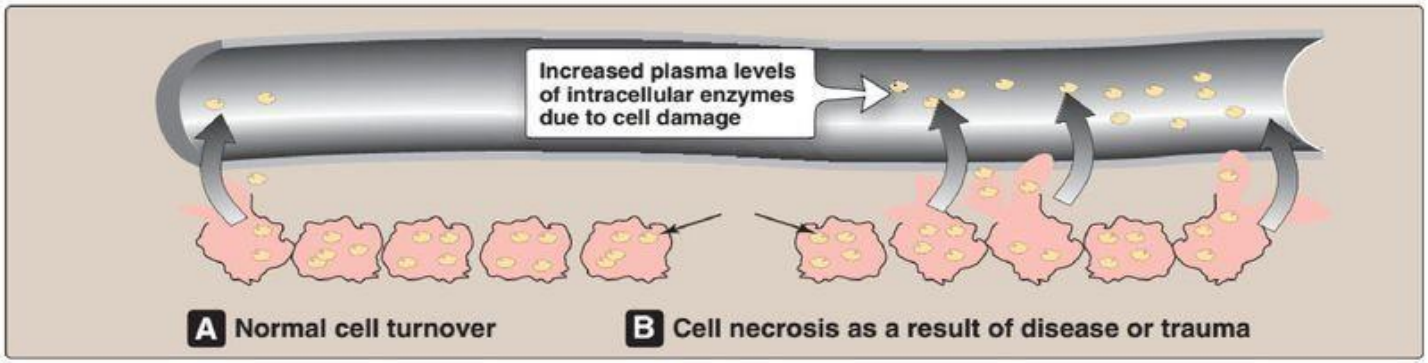
2. Large number of enzyme species are released from cells during normal cell turnover.

These enzymes almost always function intracellularly and have no physiologic use in the plasma.

Enzymes in clinical diagnosis

- In healthy individuals, the levels of these **enzymes** are fairly **constant**, and represent a **steady state** in which the rate of release from damaged cells into the plasma is **balanced** by an **equal** rate of **removal** of the enzyme protein from the plasma.

- The presence of **elevated enzyme** activity in the plasma may indicate **tissue damage** that is accompanied by **increased** release of intracellular **enzymes**.



Clinical Applications of Enzymes

Due to their diverse properties, enzymes find many uses in therapeutics, clinical analysis and disease diagnostics.

e.g. hydrolytic enzymes, such as proteases, pronase and RNases → are used in debridement of wounds.

Streptokinase → useful in clearing blood clots that occur in MI.

Plasma enzymes as diagnostic tools

- Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma.
- The activities of many of these enzymes are determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle and other tissues.
- Some enzymes show relatively high activity in only one or a few tissues.
- The presence of ↑ levels of these enzymes in plasma thus reflects damage to the corresponding tissue.

The enzyme *alanine aminotransferase ALT* is abundant in liver. Thus, elevated levels of ALT in plasma signals possible damage to hepatic tissue.

The plasma levels of *creatine kinase (CK)* are commonly determined in the diagnosis of myocardial infarction.

Clinical Enzymology

Is a branch of biochemistry dealing with the diagnostic value of enzyme estimation in serum and tissues in diseases.

Diagnostic Significance of enzymes

- Enzymes can act as diagnostic markers of underlying diseases.
- Enzymes can also act as reagents for various biochemical estimations and detections.

Enzymes as diagnostic markers

- 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 for these enzymes include; lipoprotein lipase, pseudo cholinesterase, and the proenzymes of blood coagulation and blood clot dissolution. The majority of these enzymes are synthesized in and secreted by the liver.

Enzymes as diagnostic markers

- Non-functional plasma enzymes (Cell derived enzymes): Plasma also contains other enzymes that perform no known physiologic function in the blood.

- These 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.

Units of serum enzyme activity

International Unit- One IU is defined as the activity of the enzyme which transforms one micro mole of substrate in to products per minute per liter of sample under optimal conditions and at defined temperature. It is expressed as IU/L.

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

Serum enzymes in heart disease

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

Enzyme diagnosis is necessary for MI, because:

25-30% of MI are not diagnosed clinically and by ECG.

Serum enzymes in Acute Myocardial Infarction

A. Commonly done :

- Troponins T and I
- CK creatine kinase
- Myoglobin

B. Other enzymes which have been studied but not commonly done:

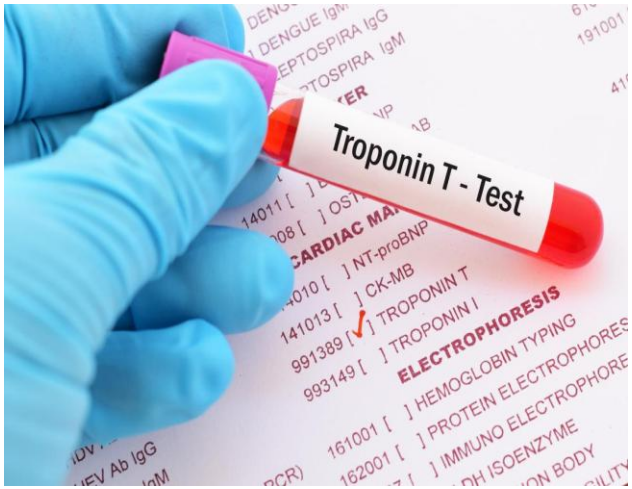
- GOT glutamate oxaloacetate transamines.
- LDH Lactate deydrogenase
- α- glutamyl transpeptidase (GGTP).
- Histaminase
- Pseudo-cholinesterase

Troponins T and I

- Troponin is a protein released from myocytes when irreversible myocardial damage occurs.
- It is highly specific to cardiac tissue and accurately diagnoses myocardial infarction with a history of ischemic pain or ECG changes reflecting ischemia.
- Cardiac troponin level is dependent on the infarct size, thus providing an indicator for the prognosis following an infarction.

- Cardiac troponins T and I are highly sensitive and specific for cardiac damage. Troponin I and T are of equal clinical value.

- Serum levels increase within 3-12 hours from the onset of chest pain, peak at 24-48 hours, and return to baseline over 5-14 days.



- Troponin levels may not be detectable for six hours after the onset of myocardial cell injury. The most sensitive *early marker* for myocardial infarction is myoglobin.

- Troponin levels should be measured at presentation and again 10-12 hours after the onset of symptoms. When there is uncertainty regarding the time of symptom onset, troponin should be measured at twelve hours after the presentation.

Creatine Kinase (CK or CPK)

- It’s 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.
- Small amounts are found in lung, thyroid and adrenal gland.
- Catalyzes the transfer of phosphate between creatine and ATP/ADP.

Creatine –P + ADP $\xrightarrow{CK}$ Creatine + ATP

- Diagnosis: for MI and muscle diseases. But remains normal in patients with liver diseases.
- Normal value: 4-60 IU/L
- After MI, S. level is found to increase within 3-6hrs, reaches a peak level in 24-30hrs, and returns to normal level in 2-4 days (usually in 72 hrs).
- There is 50% loss of S.CK activity after 6hrs at room temperature. Hence all determination of S.CK activity should be done on fresh blood samples.

Myoglobin as Cardiac marker

- One of the earliest markers is myoglobin, which is very sensitive but in certain clinical settings, lacks specificity.

- It’s level rises within 4 hours of infarction falsely high levels may be observed in patients of renal failure or patients having muscle injuries.

Serum enzyme in GI tract diseases

1- S. Amylase

2- S. lipase

- ✓ Amylase and lipase are key digestive enzymes.
- ✓ The pancreas, a glandular organ that sits behind the stomach, produces both of these enzymes.
- ✓ Amylase helps your body break down starch.
- ✓ Lipase helps your body digest fats.

S. Amylase

- Amylase are a group of hydrolases that split complex carbohydrates (breaks down starch and glycogen to maltose). They are digestive enzymes.

- Normally small amounts of amylase are presents in the blood, normal range of S. amylase is (40 – 140 IU/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.

- Estimation of plasma amylase activity is mainly requested to help in the diagnosis of acute pancreatitis , in which the plasma activity may be very high. However , it may also be raised in association with other intra – and extra abdominal conditions which cause similar acute abdominal pain.

S. lipase

- S. lipase assay is more specific in pancreatic disorder and remains raised for longer periods.
- N. value: 9-20 IU/L
- Levels as high as 2800 IU/L are seen in acute pancreatitis. Also reported high in perforated duodenal and peptic ulcers and intestinal obstruction.
- Moderate increase of S. lipase were found in about 1/3 patients with cirrhosis.

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*.

<u>These include:</u>

1. Aspartate aminotransferase (AST)
2. Alanine aminotransferase (ALT)

Amino Transferases

AST is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes and erythrocytes in decreasing order of concentration.

Normal Level (0 – 41 IU/L)

ALT is found primarily in the liver.

Normal Level (0 – 45 IU/L)

The aminotransferases are normally present in the serum in low concentrations. These enzymes are released into the blood in greater amounts when there‘s damage to the liver cell membrane resulting in increased permeability.

2. Enzymes that reflect cholestasis

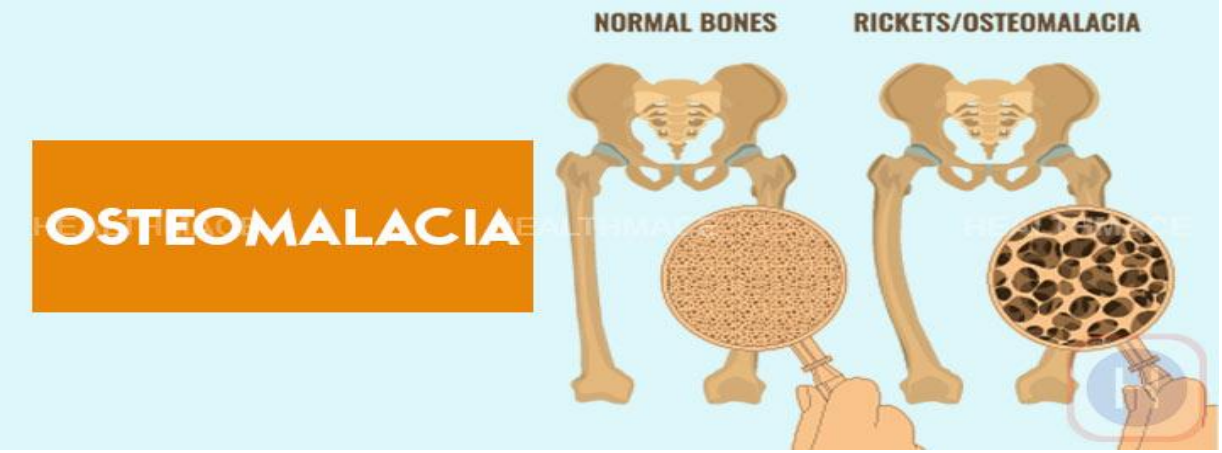
The activities of three enzymes ;

1) Alkaline phosphatase
2) 5’-nucleotidase, and
3) ɣ- Glutamyl transpeptidase (GGT) , are usually elevated in cholestasis.

Alkaline phosphatase 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 of epithelial cells.

Serum enzymes in bone diseases

1. Alkaline Phosphatase: Rises in Rickets, osteomalacia, hyperparathyroidism and in Paget's disease.



- ALP is a most valuable index of osteoblastic activity.
- The (ALP) are a group of enzymes which hydrolyze in phosphates at high pH .

- ALP are present in most tissues but are in particularly high concentration in the osteoblasts of bone, the hepato biliary tract , the intestinal epithelium , the renal tubules and the placenta.

- It is probably important for calcification of bone.

- (ALP) is activated by magnesium ions(Mg), manganese ions (Mn) and the cobalt ions (Co).

- Plasma (ALP) in adults is mainly derived from bone and liver, the proportion due to the bone fraction is increased when there is increased osteoblastic activity.
- The normal range is (21-92 IU/L)for adults, and
- (71-142 IU/L) for children.

2. Acid Phosphatase: Highly increased in bony metastasis of carcinoma prostate.

Serum enzymes in muscle disease

1- Transaminases S.GOT/S.GPT

2- Aldolase

3- Creatine kinase

Causes of raised plasma AST and ALT

1- markedly raised levels;

a- Circulatory failure with shock and hypoxia

b- Acute viral or toxic hepatitis.

c- Myocardial infarction.

Causes of raised plasma AST and ALT

2- Moderately raised levels;

a- Cirrhosis.

b- Cholestatic jaundice.

c- Skeletal muscle disease.

d- After trauma or surgery.

e- Severe hemolytic anemia.

f- Liver congestion secondary to congestive heart failure.

Enzymes which are useful in malignancies

Enzyme Assay

- S. ACP
- S. ALP
- S. LDH
- B-GLucuronidase

Used in malignancies

- Cancer of prostate with or without metastasis
- Osteoblastic metastasis in bones
- In widespread malignancies- Advanced leukemia
- Cancer of urinary bladder.