DIAGNOSTIC APPLICATION OF ENZYMES

INTRODUCTION:

- Enzymes occurrence in our body help us in the diagnosis of many diseases.
- Enzyme activities in biological fluids (serum/plasma) is of great clinical importance.
- Enzyme in the circulatory system are divided into two groups:
    1. Plasma functional enzymes
    2. Non-plasma functional enzymes

PLASMA FUNCTIONAL ENZYME:

- Enzymes that are present in plasma and have specific function are called plasma functional enzyme.
- Activities of these enzymes are higher in plasma than tissues.
- The are mostly synthesized in liver and enter the circulation.

e.g. lipoprotein lipase, plasmin, thrombin, choline, esterase, ceruloplasmin etc.

PLASMA FUNCTIONAL ENZYME:

- Impairment of liver function often leads to fall in the activities of plasma function enzyme.

e.g. deficiency of ceruloplasmin in Wilson disease.

Non Functional Plasma Enzymes

- Present in plasma in very low concentration in comparison to tissue.
- Have no known physiological function in blood.

- Their substrate is absent in plasma and appear incidentally in blood.

- Increased levels of non functional plasma enzymes in plasma indicates tissue damage.

- These enzymes can be used for diagnosis.

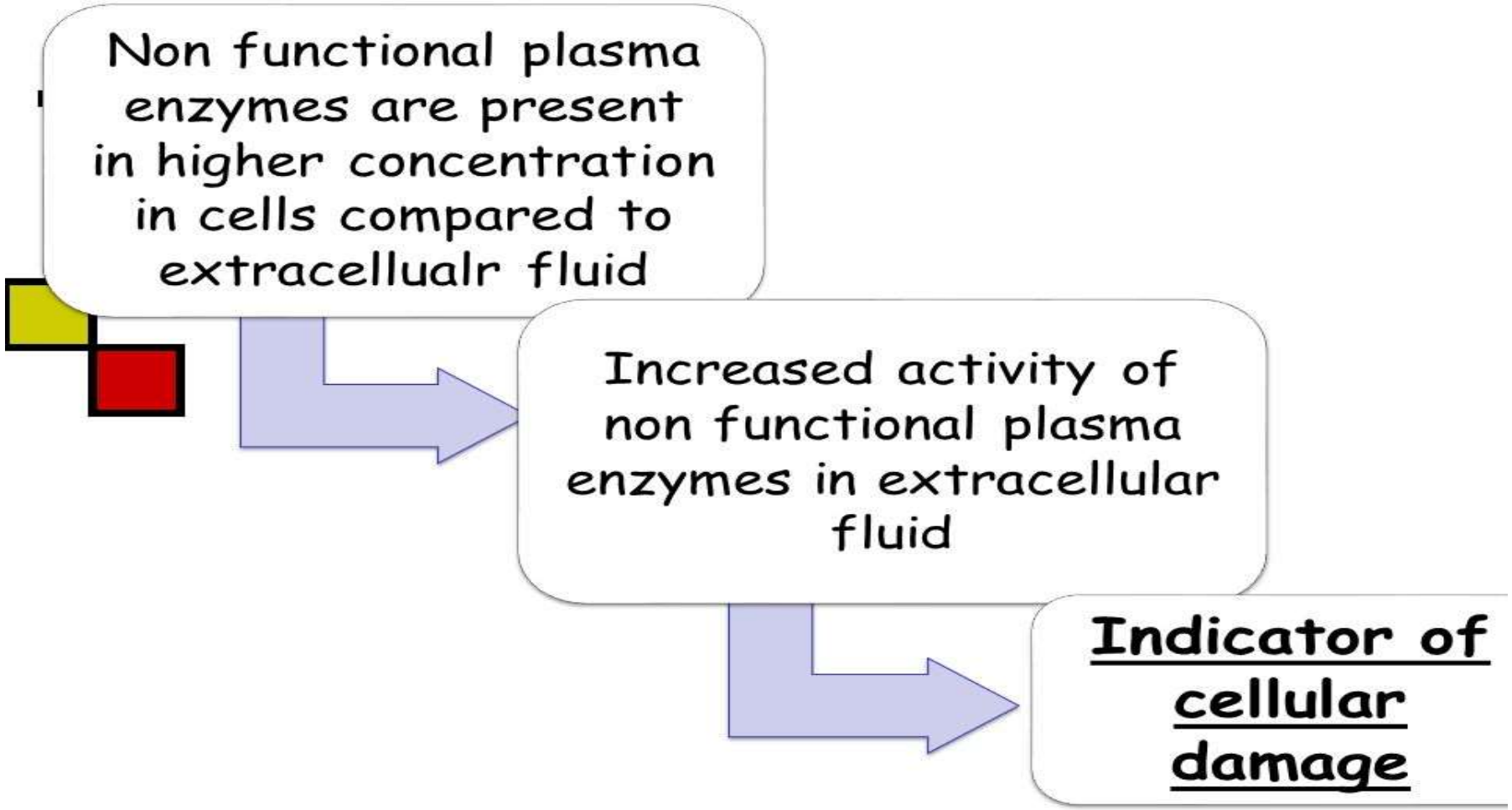
Source of non functional Plasma enzymes

Cell Damage:

e.g. Myocardial infarction and viral hepatitis.

Obstruction of Normal pathways:

e.g. Obstruction of bile duct increases alkaline phosphatase.


Non functional plasma enzymes are present in higher concentration in cells compared to extracellualr fluid
Increased activity of non functional plasma enzymes in extracellular fluid
Indicator of cellular damage

Medical importance of non functional enzymes.

- Measurement of non functional enzymes is important for:

  Diagnosis of diseases;

- As disease of different organs cause elevation of different plasma enzymes.

  Prognosis of the disease;

- To follow up of the treatment by measuring plasma enzymes before and after.

Examples

- Creatine kinase
- Lactate dehydrogenase
- Transaminase
- Acid phosphatase
- Amylase
- Alkaline phosphatase

Amylase

- It is an enzyme that catalysis the hydrolysis of starch into simpler compounds.

Pancreas

- When pancreas is damaged amylase is released into the blood.
- A significant amount of serum amylase is excreted in the urine so the rise in serum amylase is reflected in the rise of urine amylase activity.
- It is also used in detecting the development of complications following acute pancreatitis like pseudocyst, ascites, pleural effusion etc.
- Any lesion to the salivary gland due to infection(mumps) etc may lead to high amylase activity in the serum.

Aspartate Aminotransferase

- It is an enzyme involved in transamination reactions.
- These enzymes are found in most tissues but specifically in skeletal muscle, cardiac muscle, liver and kidney.
- They are useful in the diagnosis of myocardial infarctions.
- Elevated AST level is indicative of damage to myocardium.

Alanine Transaminase

- An enzyme that catalyze the reversible transfer of an amino group from alanine to alpha ketoglutarate to form pyruvate and glutamate.
- Found in high concentration in liver cell.
- Hence markedly raise activity indicate severe liver disease.
- Usually viral hepatitis or toxic liver necrosis.

Alkaline Phosphatases

- Alkaline phosphatase (ALP) refers to a group of phosphomonoesterases that hydrolyze phosphate esters with optimum in vitro activity at a pH of 10.
- Sources

It is mostly produced in your liver, but some is also made in your bones, intestines, and kidneys. In pregnant women, ALP is made in the placenta.

Clinical Significance

- The majority of sustained elevated ALP levels are associated with disorders of the liver or bone, or both. Therefore, these organ systems are of prime consideration in the differential diagnosis.
- An ALP test requires a health professional to draw a small sample of blood from your arm.

Alkaline phosphatase

Adults:	25-100 units per liter (U/L) or 0.43-1.70 microkatals/liter (mckat/L
Children	Less than 350 U/L or less than 5.95 mckat/L

High values

Very high levels of ALP indicates following diseases :

- Hepatitis (inflammation or infection of the liver)
- Cirrhosis (scarring of the liver)
- Cholecystitis (inflammation of the gallbladder)
- Blockage of bile ducts (from gallstone, inflammation, or cancer
- Rickets: Weakening of bones due to deficiency of vitamin D, calcium, or phosphate.
- Osteomalacia: Softening of bones due to vitamin D deficiency.
- Paget's disease : Disorder causing major problems with bone destruction and regrowth.

Low values

- **Lower** than normal **ALP levels** in your blood is rare, but can indicate malnutrition, which could be **caused** by celiac disease or a deficiency in vitamins and minerals.

PROGRESSION OF LIVER DAMAGE

HEALTHY LIVER

A healthy liver is able to perform its normal functions effectively, e.g. aiding digestion and breaking down harmful drugs and poisons.

FIBROTIC LIVER

Continuous inflammation of the liver caused by hepatitis C can lead to fibrosis – the formation of scar tissue within the liver.

CIRRHOTIC LIVE

Extensive scarring can block the flow of blood through the liver and cause liver function to deteriorate over time - this is called cirrhosis.

LIVER CANCER

Hepatitis C is a leading cause of liver cancer – the formation of a malignant tumour in the liver.

Source: Boehringer Ingelheim

Acid Phosphatase

- Name of family in which many enzyme are present.
- Non functional plasma enzyme.
- An enzyme that act to liberate phosphate under acidic condition.
- Made in liver, spleen, bone marrow, kidney, RBC's and prostate gland.
- This enzyme is 100 time more produce in prostate gland than other organ of body.
- Normal level of acid phospatase is 0.5 to 4 KA.

Diagnostic Applications

Different form of acid phosphotase are found in different organ and their level are used to evaluate the success of certain diseases.

Prostrate cancer.

- Acid phospatase in prostrate gland(gland in male reproductive system) is prostatic specific acid phospatase.
- When tumor appear in prostate gland, level of Acid Phospatase increase in blood. Test is not for the screening of cancer, level of enzyme rises when prostrate cancer has metastasized.

Contin…….

Bone disease.

- Acid phosphatase present in bones is Tartrate resistance acid phosphatase.
- When level of this enzyme increase in blood this indicate that Bone resorption disease is present.
- Bone resorption is that disease in which osteoclasts break down tissues in bones and release minerals.

As Acid phosphatase enzyme is present in many organs of body, damage of the tissues of these organs moderately increase in level of this enzyme.

Lactate Dehydrogenase

Enzyme that catalyze the conversion of sugar into energy.

- Found in liver, heart, pancreas, kidney.
- It is released during tissue damage.
- It is a marker of common injuries and diseases.

Diagnostic Application

- Used for the diagnosis of;
  - Myocardial infraction
  - Infective hepatitis
  - Leukemia
  - Muscular dystrophy

Cont…

- LDH have five isoenzyme
- **LDH 1:** Heart and RBC
- **LDH 2:** WBCs
- **LDH 3:**Lungs
- **LDH** 4:Kidneys
- **LDH 5**: Liver and skeletal muscles.

Test: A test is performed in the labs.

Result

High LDH Level:

High level of LDH indicate some form of tissue damage(For instance a person with high level of LDH will indicate muscle damage).

Low LDH Level:

It is very rare for a person to have low LDH level

Creatine Kinase

- Necessary for muscle cells of the body to achieve their different chemical reactions.
- Increased level of CK in blood indicate a muscular disorder
- Found in brain and skeletal muscles.

Diagnostic applications

- Used for diagnosis of damage to muscles including heart muscles.

Cont…

- CK has three isoenzymes:

CK-MM: Found in skeletal muscles.

Its level rises if you have muscle damage in your heart, brain or skeleton.

CK-MB: Found in the heart.

Its level rises after heart attack, inflammation of heart muscles, muscular dystrophy.

CK-BB: Found mostly in brain.

Its level rise if you have brain injury, meningitis, abnormal cell growth, stroke and hypothermia.

Gamma-Glutamyl Transpeptidases

Gamma-Glutamyl Transpeptidases (also called gamma-glutamyl transferase or GGT) is a transferase that catalyzes the transfer of gamma-glutamyl functional group from molecule such as glutathione to an acceptor molecule that may be an amino acid, a peptide or water.

Nomenclature

The name gamma-glutamyl tranferase is preferred by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology.

The older name is gamma-glutamyl transpeptidase (GGTP).

Site

Highest tissue activity of this enzyme is found in kidneys, but activity is relatively high in liver, lungs and pancreas. Some activity is present in intestinal mucosa, thyroid gland and spleen. Normal heart contains very little gamma-GTP.

Normal Value

Normal serum activity has been shown to be:

Men: 10 to 47 IU/L

Women : 7 to 30 IU/L

Medical Applications

- **Gamma-glutamyl transpeptidases has been proposed as a useful test in myocardial infarction in later stages because several investigators recently have demonstrated increases in serum gamma-GTP in acute myocardial infarction.**
- **GGTP is used as a diagnostic marker for liver diseases such as obstructive jaundice and hepatitis. Elevation in GGTP are typically seen in patients with chronic hepatitis infections.**

Continue

- **It is a sensitive diagnostic marker for the detection of alcoholism. It is also found elevated in alcoholics and is the most sensitive indicator in alcoholics.**

- **It is also used as a diagnostic marker for pancreatic diseases because its increased serum activity is also seen in pancreatic diseases.**

Other Function

GGTP is also involved in glutathione metabolism by transferring the glutamyl functional group to a variety of acceptor molecules including water, certain L-amino acids and peptides. The general reaction is:

(5-L-glutamyl)-peptide + amino acid ⇌ peptide + 5-L-glutamyl amino acid

Decreased Plasma Enzyme Activity

- Plasma activities of certain enzymes may be lower than normal due to decreased enzyme synthesis or congenital deficiency. This also help us in diagnosis of certain diseases.

Cholinesterases

- Cholinesterases are enzymes which hydrolyse esters of choline to give choline and acid.

There are two types of cholinesterases:

True cholinesterase : responsible for the destruction of acetyl choline (neurotransmitter) at neuromuscular junction and is found in nerve tissue and RB cells.

Pseudo cholinesterase : found in various tissues such as liver, heart muscle and intestine. This type is present in plasma.

Normal value :

Normal value of pseudo cholinesterase is 2.17 to 5.17 IU/ml.

- Organophosphorus compounds

These are organic compounds containing phosphorus. These are toxic compounds used for pest control. Their absorption in humans cause poisoning.(neuromuscular damage, muscle weakness, slow breathing)

1)**Cholinesterase test** : it is used to detect and diagnose organophosphate pesticide exposure or poisoning.

- To cheque the level of acetyl cholinesterase in RBC and pseudo cholinesterase in plasma.
- Upon exposure AChE and PChE activity can fall to about 80% of normal before any symptoms appear, of poisoning.
- This decrease in activity indicates excessive absorption of organophosphorus compounds.

2)Cholinesterase test

- It is used in testing for succinylcholine sensitivity.

Succinylcholine : neuromuscular blocking agent used as muscle relaxant usually before surgeries.

- About 3% of people have low activity levels of pseudo cholinesterase due to an inherited deficiency.
- Pseudo cholinesterase enzyme hydrolyses succinylcholine.
- Its deficiency can result in higher levels of intact succinylcholine molecules reaching receptors in the neuromuscular junction, causing the duration of paralytic effect to continue for as long as 8 hours.

- Reduced cholinesterase levels can also be caused by chronic liver disease, renal disease , malnutrition and some cancers.

Ceruloplasmin

- Copper containing protein found in blood.
- It carries 10% of the copper in blood.
- Normal Ceruloplasmin level is 1-4.
- **Abnormalities:**
- **High Level: Its level is high during pregnancy, cancer and several mental conditions such as Alzheimer's disease.**
- **Low Level: Its found abnormally in low amount in case of Wilsons disease.**

Glucose-6-phosphate Dehydrogenase:

- It is a cytoplasmic enzyme that is distributed in all cells.
- The most common medical problem associated with G6PD deficiency is hemolytic anemia, which occur when the RBCs are destroyed faster than the body can replace them.