Clinical enzymology

Enzymes

- Biological catalysis
- Very efficient – can increase reaction rates at the order of x 10
- All are proteins - so liable to denaturation
- Specific to substrates
- Partly specific to tissues
- Assay by measure of rate of specific reaction catalyzed by that enzyme

Measurements of the activity of enzymes in plasma are of value in the diagnosis and management of a wide variety of diseases.

Groups of enzymes:

A- Present in high concentration in cells but low concentration in plasma
   - Important in diagnosis and prognosis (in the initial state, diseases during recovery and recovery and repair)
   - Function inside cell and no function in blood e.g. (ALP, ACP, CK, transaminase and lipase)
B- Present in high concentration in plasma and low concentration in cells
   - Less important as diagnostic enzymes, function outside the cell (in the blood) e.g. blood coagulation enzymes.

Non-specific causes of raised plasma enzyme level

1- Physiological
   - New born (AST and CK elevated immediately after labour due to muscular contraction during delivery and also elevated during and after strenuous exercise)
   - Childhood (ALP of bony origin)
   - Pregnancy (ALP placental increase during last trimester due to placental isoenzyme)
2- *Enzyme induction by drugs* (as gamma glutamyl transferase GGT is elevated by alcohol, anti-convulsants phenytoin and phenobarbitone may induce microsomal enzyme)

3- *Artfactual alteration of enzyme* (haemolysed sample)

**Low enzyme levels**

1- Reduced synthesis  
2- Congenital deficiency (ChE)

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### Enzymes routinely measured

<table>
<thead>
<tr>
<th>NAME OF THE ENZYME</th>
<th>PRESENT IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate Amino transferase (AST)</td>
<td>Heart and Liver</td>
</tr>
<tr>
<td>Serum glutamate-oxaloacetate transaminase (SGOT)</td>
<td>Heart and Liver</td>
</tr>
<tr>
<td>Alanine Amino transferase (ALT)</td>
<td>Heart and Liver</td>
</tr>
<tr>
<td>Serum glutamate-pyruvate transaminase (SGPT)</td>
<td>Heart and Liver</td>
</tr>
<tr>
<td>Alkaline Phosphatase (ALP)</td>
<td>Bone, intestine and other tissues</td>
</tr>
<tr>
<td>Acid Phosphatase (ACP)</td>
<td>Prostate</td>
</tr>
<tr>
<td>(\gamma) glutamyl Transferase ((\gamma) GT)</td>
<td>Liver</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td>Muscle Including cardiac muscle</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH)</td>
<td>Heart, liver, muscle, RBC</td>
</tr>
<tr>
<td>(\alpha) Amylase</td>
<td>Pancreas</td>
</tr>
</tbody>
</table>

**Isoenzymes:**

Different forms of enzyme that catalyse the same reaction but differ in their physiological and chemical properties or physically distinct form of same catalytic activity may also present in different tissues of same organism because of genetically determined aminoacid sequence.
Diagnostic significance of isoenzymes

1- Lactate dehydrogenase (LDH)

Pyruvate $\rightleftharpoons$ lactate

<table>
<thead>
<tr>
<th>LDH</th>
<th>Subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HHHH</td>
</tr>
<tr>
<td>2</td>
<td>HHHM</td>
</tr>
<tr>
<td>3</td>
<td>HHMM</td>
</tr>
<tr>
<td>4</td>
<td>HMMMM</td>
</tr>
<tr>
<td>5</td>
<td>MMMM</td>
</tr>
</tbody>
</table>

The way of separation of isoenzymes by electrophoresis each one have different no. of charged amino acids

<table>
<thead>
<tr>
<th>Isoenzyme name</th>
<th>Composition</th>
<th>Composition</th>
<th>Present in</th>
<th>Elevated in</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH1</td>
<td>$(H_4)$</td>
<td>HHHH</td>
<td>Myocardium, RBC</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>LDH2</td>
<td>$(H_3M_1)$</td>
<td>HHHM</td>
<td>Myocardium, RBC</td>
<td></td>
</tr>
<tr>
<td>LDH3</td>
<td>$(H_2M_2)$</td>
<td>HHMM</td>
<td>Kidney, Skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>LDH4</td>
<td>$(H_1M_3)$</td>
<td>HMMM</td>
<td>Kidney, Skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>LDH5</td>
<td>$(M_4)$</td>
<td>MMMM</td>
<td>Skeletal muscle, Liver</td>
<td>Skeletal muscle and liver diseases</td>
</tr>
</tbody>
</table>

LDH1 presents in heart and increases in case of myocardial infarction, LDH2, LDH3 in malignancy especially leukemia, LDH4 and LDH5 in liver and muscle diseases.

2- Alkaline phosphatase (ALP): has many isoenzymes most common in liver and bone and in placenta as well.
- If we have a male with high alkaline phosphatase that shows that there is a malignancy. But in case of high alkaline phosphatase in a pregnant women that shows she has a normal because ALP found in placenta as we mention.
3-Creatin kinase (CK) or Creatin phosphokinase (CPK): there are 3 isoenzymes MM, MB, and BB. MB type is specific for heart and it increases in case of myocardial infarction (M.I). MB type account normally 20% of total CK. It present in muscle and brain also. MM-type in muscles

Causes of CK elevation:
  a) Muscle dystrophy
  b) Trauma
  c) Myocardial infraction
  d) Sever exercise and intramuscular injection

After car accident or fall from high, an increase in CK does not indicate myocardial infraction because it may trauma or muscle damage so we should check for MB type.

4-Acid phosphatase (ACP): in body there are 2 types of isoenzymes which most commonly used for a diagnosis of prostatic disease

Factors caused an increase in the rate of release enzyme:

1- necrosis or sever damage of cell (ischemia or toxic)
2- High rate of cell turnover (absorption)
3- Increase of concentration of enzyme within the cell (e.g. GGT by alcohol)
4- Obstruction e.g. Amylase may be regurgitation in blood

The choice of enzyme test depend upon
- Nature of disease
- Site of disease

The factors important in making this choice are:
1- Sensitivity: the ability to detect small amount of tissue damage by measure enzyme activity
2- Specificity: ability to identify which tissue has been damaged
3- Time course of enzyme elevation: enzyme activity should raise soon and remain raised after the onset of disease depend on T1/2 (half-life) and duration of enzyme release
4- Technical factor: accurate, precise, easy and expensive
How the damage site located?
Damage of organ causes release of large amount of enzyme into blood stream. Lack of specificity is more of problem since enzymes are widely distributed this problem can partly overcome by measuring several enzyme activities or by study of isoenzyme.

Enzyme unit:
International unit (I.U): is the amount of enzyme which will catalyse the conversion of (1 micro mol [μmol]) of substrate per minute per liter at certain temperature and PH.

- **Katal (catalytic activity)** is defined as the number of mole of substrate transformed per second per litre of sample.

  Katal = 16.7 mol/sec

Assessment of cell damage and proliferation plasma enzyme level depends on:

- Rate of enzyme release from damaged cells which in turn depend on the rate at which damage is occurring and extend of cell damage

  In the absence of cell damage the rate of release depend on:
  
  a) Rate of cell proliferation 
  b) The degree of induction of enzyme synthesis 
  c) Drug affect synthesis of enzymes 

  These factors are balanced by the rate of enzyme clearance from the circulation

For example:

- Viral hepatitis: acute cell damage may cause high enzyme activity that return to normal after resolution
- Viral hepatitis attack cell membrane

  In normal condition  enzyme level 20-50 I.U
  In damaged condition  100-200 I.U

  If liver affected by much more rate of damage as “cirrhosis” plasma activity may be slightly elevated or upper normal level
- In severe liver disease plasma activity may even fall as no. of hepatocytes is grossly reduced (level of enzyme is normal or reduced) most enzymes removed from the circulation by catabolizing by plasma protease before taken up by endoplasmic reticulum (E.R)

- Some enzyme are cleared by kidney due to their low molecular weight In healthy individual, fairly constant enzyme level and high level as in case of myocardial infarction, CK, GOT and LDH are elevated Renal glomerular impairment may delay the rate of fall of those plasma enzymes cleared through kidney, as amylase increase in renal disease because of impaired release rather than pancreatic or salivary diseases

- As damage increase, enzyme level of cell increase
- In case of liver failure there will be no enzyme clearance so enzyme level is elevated.

**Enzyme in diagnosis:**

To detect organ involved either electrophoresis or estimated by two enzymes related to same organ.

- Cellular distribution of enzyme as ALT present in cytoplasm of hepatocyte while AST in mitochondria of hepatocyte thus in acute disease as hepatitis the first enzyme elevated is ALT, while in chronic disease, AST is elevated as well as ALT but in lesser extent.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase ALP ALP</td>
<td>Liver, bone, placenta, RBC</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>Prostate</td>
</tr>
<tr>
<td>Amylase</td>
<td>Salivary gland and pancreas</td>
</tr>
<tr>
<td>Gamma glutamyl transferase(GGT)</td>
<td>Alcoholic liver</td>
</tr>
<tr>
<td>Aspartate transferase AST (GOT)</td>
<td>Liver, heart</td>
</tr>
<tr>
<td>Alanine transferase ALT(GPT)</td>
<td>Liver</td>
</tr>
<tr>
<td>Creatin kinase CK (CPK) Creatin kinase CK</td>
<td>Heart, muscle, brain</td>
</tr>
</tbody>
</table>


**What is Myocardial Infarction?**

- Myocardial ischemia results from the reduction of coronary blood flow to an extent that leads to insufficiency of oxygen supply to myocardial tissue
- When this ischemia is prolonged & irreversible, myocardial cell death & necrosis occurs ---this is defined as:
  - *myocardial infarction*
    - is the death & necrosis of myocardial cells as a result of coronary prolonged & irreversible ischemia

**biochemical Changes in Acute Myocardial Infarction**
*(mechanism of release of myocardial markers)*

Diagnosis of Myocardial Infarction

SHOULD depend on **THREE** items (as recommended by **WHO**)

1- Clinical Manifestations
2- ECG
3- Biochemical Markers

Types of **Biochemical Markers for Myocardial Infarction**

1-Cardiac Enzymes (isoenzymes):
   - Total CK
     - CK-MB activity
CK-MB mass

- **Total LDH**
  - LDH1
  - LDH2 (LDH1 > LDH2)

- **ASP (GOT)**

2- **Cardiac proteins:**
  - Myoglobin
  - Troponins

**Duration of elevation**
In myocardial infarction 3 enzymes are elevated, but in different periods.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Starts to elevate</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK(CPK)</td>
<td>4 h</td>
<td>24-48 h</td>
</tr>
<tr>
<td>AST</td>
<td>12-24 h</td>
<td>24-72 h</td>
</tr>
<tr>
<td>LDH</td>
<td>12-24 h</td>
<td>2-10 days</td>
</tr>
</tbody>
</table>

**Case study:**

**Myocardial infarction (M.I)**

**Case 1:**
Patient with M.I was taken to hospital after 2 h blood sample is prepared, which of the following enzymes are elevated, CPK, AST, LDH?

*Answer:* Non of above

**Case 2:**
Patient with M.I was taken to hospital after 2 days blood sample is prepared, which enzyme is elevated, CPK, ASP, LDH?

*Answer:* All of them
Case 3:

Patient with M.I was taken to hospital after 12 days blood sample is prepared, which enzyme is elevated, CPK, ASP, LDH

Answer: non of the above, if CPK is elevated after 12 days that means there is another M.I after the recovery from first one

AST $5-20$ I.U/liter