***Dr. Adnan F. Alnajjar* Clinical enzymology**

 Enzymes are protein in nature; catalyze reaction which would not take place if it is not present. Present in every cell. About 1500 enzymes. Specific enzyme required for each step.

**Groups of enzymes:-**

1. Present in high conc. In cell but low conc. in plasma. Important in diagnosis & prognosis (in the initial stage & in the disease & during recovery & repair) function inside cell & no function in blood.

Examples: ALP, ACP, Transaminases, CK, lipase……. .

1. Present in high com. in plasma & low in cells. less Important as diagnostic enzymes. Function outside the cell ( in the blood ) .

Examples: blood coagulation enzymes, Renin angiotensin system.

**Non-specific causes of raised plasma enzyme levels** :-

 Newborn ( AST )

1- Physiological Childhood (ALP)

 Pregnancy (ALP)

2- Enzyme induction by drugs (GGT by alcohol).

3- Artefactual elevation of enzyme (haemolysed sample).

**Low enzyme levels:-**

1- Reduced synthesis.

2- Congenital deficiency (CHE)

**Isoenzymes**:

Different from of an enzyme, catalyze the same reaction but differ in it's physico-chemical properties. It can separated by electrophoresis, chromatography & by chemical means e.g. LD…LD1, LD2…..

**Factors regulate enzyme activity**:-

1- Rate of release of the enzyme from cells.

2- Volume of distribution of enzyme in E.C.F..

3- Rate of removal of enzyme from plasma by catabolism or excretion.

The plasma half lives of enzymes is 10 hours\_ 5 day.

Enzymes in Circulation

Uptake into cells Excretion in

of lymphoreticular system urine

 *Rate of enzyme removal from circulation*

4- Presence in the plasma of factors which may affect the procedure of assays e.g. inhibitor or activator.

**Factors caused increase in rate of release of enzyme:**

1. Necrosis or server damage to cells

 Ischemia or toxic substances release of enzyme (especially those in cytoplasm).

1. ↑ Rate of cell turnover :

 E.g. during active growth in first year of life or puberty (ALP) or tissue repair (healing fracture) → ↑ ALP or in malignant disease.

1. Increased conc. of enzyme within the cell :-

 Like induced by certain disease or drug (GGT by alcohol)

1. Duct obstruction :

 E.g. amylase may be regurgitated into blood.

**Selecting plasma enzyme test :-**

Damage of organ causes release of large amount of enzymes into the blood stream.

The choice of test depends on nature & site of the disease & the factors important in making the choice are:-

1- Sensitivity: the ability to detect small amount of tissue damage by measuring enzyme activity (it can be very difficult to make a diagnosis when tissue damage is small).

2- Specificity: ability to identify which tissue has been damaged.

3- Time – course of enzyme elevation: enzyme activity should rise soon & remain raised for a proper period after the onset of disease.

It depends on (half-life, duration of enzymes release).

4-Technical factors: accurate, precise, easy & inexpensive.

 So how is the site of damage located?

Lock of specificity is more of problem since enzymes are widly distributed, this problem can partly overcome by measuring several enzyme activities or by the study of Isoenzymes.

**Enzyme units:**

Conc. & activity of enzyme are usually proportional to one another.

I.U. (international unit): is the amount of enzyme which will catalyze the conversion of 1 Mmol of substrate per minute.

Some U.K hospital laboratories use katals (1 Katal = 6 × 10­7 I.U.)

Katals (mol / second).

**Some Clinically important plasma enzymes:**

**Lactate dehydrogenase: (LD) or (LDH):**

It is widly distributed in heart, skeletal muscle, liver, brain, R.B.C., spleen & lung.

It catalysis the reversible inter conversion of lactate & pyruvate.

Causes of raised LD levels:

Art factual: in vitro hemolysis, on delayed separation of plasma, from cells.

Marked increase: (more than five time normal)

1- M.I. (myocardial infarction)

2- Some hematological disorders.

In pernicious anemia & leukemia very high value (up to 20 times) and lesser increase in thalassemia & haemolytic anemia.

3- Circulatory failure with shock & hypoxia

4- Renal infarction (some time rejection of renal transplant).

Moderate Increase:

1- Viral hepatitis.

2- Any malignancy.

3- Skeletal muscle disease.

4- Pulmonary embolism.

5- Infections mononucleosis.

**Transaminases :- AST (GOT ) & ALT ( GPT):**

Enzymes that are involved in the transfer of an amino group from an α-amino to an α-oxo acid . pyridoxal phosphate is the cofactor for this reaction .

a) Aspartate transferase (AST ) or glutamate oxaloacetate transaminase ( GOT ) .

b) Alanine transferase (ALT) or glutamate pyruvate trasaminase (GPT) .

AST is widly distributed with high conc. in heart, liver, skeletal muscle, kidney & erythrocytes.

ALT present in high conc. in liver & lower conc. in skeletal muscle, kidney & heart.

Causes of raised AST & ALT in plasma:-

1) Markedly raised level : ( 10 – 100 times normal )

a) viral hepatitis (ALT > AST )

b) Toxic liver necrosis ( ALT >AST )

c) Circulatory failure, with shock & hypoxia.

d) M.I. (for AST only).

2) Moderatly raised level:-

**Cholinestrate: (ChE):**

There are two related enzymes that have the ability to hydrolyse acetylcholine:

* 1. cholinesterase I (true ChE): found at nerve endings & R.B.C.
	2. cholinesterase II (pseudo ChE): present in plasma & liver.

Plasma ChE is synthesized in liver and its activity tends to reduced whenever plasma (albumin) is reduced.

Causes of low plasma chE activity:

1. physiological: in pregnancy (third trimester) & infancy.
2. Inherited: rare (abnormal ChE)
3. Acquired:

a-Diseases: liver disease, malnutrition, chronic renal failure and collagen disease.

b- Poisoning and effect of various drugs: insecticides (organophosphorus), oral contraceptive, cytotoxic drug and x-ray therapy.

**Scoline apnoea:**

Succinyl dicholine (scoline) the muscle relaxant (used in anesthesia) is hydrolysed normally by ChE.

Some patients that have abnormal ChE variants have low ChE activity in plasma, and they develop prolong apnoea (several hours) after scoline treatment.

 **Plasma patterns in diseases:**

  **(Myocardial infarction):**The enzyme estimation of greatest value in the diagnosis of myocardial infarction are AST(GOT), LD (LD1) & CK.

 Enzyme

 Activity

 20

 15

 5

 0

 1 2 3 4 5 6 7 days

**\_\_ CK-MB**

\_\_\_  **Total CK**

**\_ \_ \_ AST**

**\_\_\_\_\_ LD1(heart specific LD**)

\_ \_ \_  **LD(Total)**

|  |  |  |  |
| --- | --- | --- | --- |
| Enzyme | Start to rise (hours) | Time after infarction peak elevation (hours) | Duration of rise (days) |
| Total CK | 4-8 | 24-48 | 3-5 |
| AST (GOT) | 6-8 | 24-48 | 4-6 |
| LD(LD1) | 12-24 | 48-72 | 7-12 |

All enzyme levels normal until at least 4 hours after infarction (should be considered in taken blood sample) 4 hours after chest pain. They are raised rapidly to a peak (but give rough index of the extent of damage and as such is of some value in prognosis). Then return to normal at rates which depends on half-life of each enzyme in plasma.

A second rise in isoenzymes levels after their return to normal indicates:

1- Extension of the infarction.

2- Or the development of congestive cardiac failure (here the LD1 & CK do not increase, only AST)

Levels in angina are normal:

-If the patient is first seen after total LD has returned to normal, diagnosis may still be possible on the basis of the finding of a raised heart isoenzyme (LD1) by isoenzyme electrophoresis.

- Measurement of total CK activity is rarely helpful in the diagnosis of M. I. . If it is high when the AST & LD1 are normal, the elevation is most likely to be due to entirely to the MM isoenzyme derived from skeletal muscle, perhaps because of an intramuscular injection, recent exercise or surgery. Detection of CK-MB by isoenzyme electrophoresis may occasionally help to detect a very recent infarction, but in most cases nothing is lost by taking a later sample for AST & LD1.

Any patient suspected of having had a M. I. should be kept under observation and delay of few is not detrimental to his management. Most of CK released after M. I. is the MM isoenzyme, common to skeletal & myocardial muscle; the MM has a longer half life than the MB fraction & after about 24 hours plasma elevation of MM with undectable MB does not exclude myocardial damage as a cause of elevated total CK levels; at this time the trausaminase & LD1 pattern is usually characteristics

In most cases of suspected M. I., AST & LD1 estimations, together with the clinical & E. C. G. findings are adequate to make a diagnosis.

 **(Trauma and operation)**

Enzymes activity increase after major operation or as a result of sever trauma (road traffic accident). The changes are similar to those observed after acute. M. I. except Ck-MB is normal.

 **(Muscle disease)**

Total CK is the measurement of choice.

AST, LD, ALT may raised also.

 - levels are highest (up to 10 times normal ) in the early stages of the disease. Later, when much of the muscle has wasted, they are lower & may be even normal.

-Levels are higher following activity immediately after rest (release of CK built up in muscle during rest) than after prolonged activity.

-In detecting affected newborns, it must be remembered that levels at this age are higher than in adults.

-Similar but less marked changes are found in many subjects with myositis

 **(Liver disease)** discuss later in detail

Three patterns of altered enzyme activity may be seen:

1. Hepatocellular damage: AST & ALT (cytoplasmic enzyme).

ALT is liver specific **›** AST.

1. cholestasis :ALP, GGT (membrane associated enzyme).
2. Impaired production of certain enzyme: e. g. ChE

 **(Lung disease)**

After pulmonary embolism LD activity may increase**.**The isoenzyme pattern in serum is different in these patient from the pattern produced after M. I. However, the changes after pulmonary embolism have little diagnostic value in differentiating between pulmonary embolism and pneumonia.

 **(Enzymes in malignancy)**

1. Prostatic ACP rise in some cases of malignancy of the glands.
2. Malignancy any where in the body may be associated with a non-specific increase in LD and transaminases.
3. For follow up of treated cases of malignancy, ALP estimations are of value. Raised levels may indicate secondary deposits in the bone or liver. Liver deposit may produce an increasein transaminases and LD.
4. Tumours may produce a number of enzymes such as ALP, LD and CK-BB.

 **(Haematotological disorders)**

The extreme elevation of LD in megaloblastic anaemia and leukaemia has been mentioned before. Typically there is much less change in the level of AST than that of LD. Sever haemolysis produces changes in AST and LD.

**Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency:**

Heridotory, rare disease due to deficiency of enzyme (G6PD). There may be no clinical effects, but in other individuals the defect may cause one of the following clinical syndromes:

1. Hereditary non-spherocytic haemolytic anaemia.
2. Drug –induced haemolytic anaemia. Primaquine and the sulphanomid are example of these drugs.
3. Favism, an acute, sever haemolytic disorder which follow ingestion of the fava bean.

 **Summary**

1. Enzymes activities in cells are high. Natural decay of cells release enzymes into the plasma, where activities are measurable, but usually low.
2. Plasma enzyme estimations are most useful to detect raised levels due to cell damage.
3. Assay of few enzymes are specific for any one tissue, but isoenzymes studies may increase specificity. In general patterns of enzyme changes together with clinical findings are used for interpretation.
4. Non –specific causes of raised plasma enzyme activity include a peripheral circulatory insufficiency, trauma, malignancy and surgery.
5. Enzyme estimation are of value in:
6. **M. I.** –AST (GOT), LD isoenzyme LD1 and sometimes CK.
7. **Liver disease**-trausaminases, ALP and sometimes GGT.
8. **Bone disease** –ALP.
9. **Prostatic carcinoma**-tartrate –labile ACP.
10. **Acute pancreatitis**- α-amylase
11. **Muscle disorders**-CK.
12. **Haematological disorder** **(sever haemolysis)-**LD and AST.
13. **Organophosphorous insecticide poisoning** –ChE.

6- **Artefactual increases** may occur in haemolysed samples