Lecture: 5 **Dr. Maytham Ahmed**

Plasma Enzymes in Dignosis

Enzymes present in plasma can be classified into 2 types, they are:

1. Functional plasma enzymes
2. Non functional plasma enzyme

	Functional plasma enzymes	Non functional plasma enzymes
Concentration in plasma	Present in plasma in higher concentrations in comparison to tissue	Normally, Present in plasma in very low concentrations in comparison to tissue
Function	Have known functions	No known functions
Substrate	Their substrates are always present in plasma	Their substrates are absent from plasma
Site of synthesis	liver	Different organs .g. liver heart, skeletal muscles and brain
Effect of disease	Decrease in liver disease	Increase in different organ diseases
Examples	Clotting factors e.g. Prothrombin Lipoprotein lipase, Pseudocholinesterase	ALT, AST, CK, LDH, alkaline phosphatase, acid phosphatase and lipase

The enzyme activity in plasma may be higher than normal, due to:

1. The proliferation of cells
2. An increase in the rate of cell turnover or damage
3. An increase in enzyme synthesis (induction)
4. A clearance reduction of enzyme from plasma

The enzyme activity in plasma may be lower than normal, due to:

1. A reduced synthesis
2. A congenital deficiency

Some non-specific causes of raised plasma enzyme activities

1. Slight rises in plasma ALT and AST activities are non-specific findings in many **illnesses**.

2. Moderate **exercise**, or a large intramuscular **injection**, may lead to a rise in plasma CK.

3. Some **drugs**, such as the anticonvulsants phenytoin and phenobarbital, may induce the synthesis of the microsomal enzyme γ-glutamyl transferase (GGT), and so increase its plasma activity in the absence of disease.

4. Plasma enzyme activities may be raised if the rate of **clearance** from the circulation is reduced. In the absence of hepatic or renal disease, this may occur if, for example, the plasma enzyme forms complexes with immunoglobulins, known as a macroenzyme.

Factors affecting results of plasma enzymes assays

A. Analytical factors:

1. Substrate concentration
2. Product concentration
3. Enzyme concentration
4. Reaction temperature
5. Reaction pH
6. Presence of activators or inhibitors

B. Non-disease factors:

1. **Age:** Plasma AST activity is moderately higher during the neonatal period than in adults. Plasma ALP activity of bony origin is higher in children than in adults.
2. **Sex:** Plasma CK activity is higher in males than in women, probably in part due to their increased muscle bulk.
3. **Race/ethnicity:** Plasma CK activity is higher in black people than in white people.
4. **Physiological conditions:** Plasma ALP activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme.

Medical importance of measurement of non functional plasma enzymes activities

1. **Diagnosis** of diseases as disease of different organs cause elevation of different plasma enzymes
2. **Prognosis** of the disease and **follow up** of the treatment by measuring plasma enzymes before and after treatment

Clinical importance of some enzymes

1. Aminotransferases (ALT and AST)

The aminotransferases (ALT and AST) are enzymes involved in the transfer of an amino group from a 2-amino acid to a 2-oxoacid; they need the cofactor pyridoxal phosphate for optimal activity. They are widely distributed in the body.

A. Aspartate aminotransferase (AST)

Aspartate aminotransferase (glutamate oxaloacetate aminotransferase, GOT) is present in high concentrations in **cytosol** and **mitochondria** of cardiac and skeletal muscle, liver, kidney and erythrocytes cells. Damage to any of these tissues may increase plasma AST levels found in.

Causes of raised plasma AST activities:

1. Physiological: during the neonatal period

2. Marked increase in hepatitis and myocardial infarction

B. Alanine aminotransferase (ALT)

Alanine aminotransferase (glutamate pyruvate aminotransferase, GPT) is present in high concentrations in the cytosol of liver and, to a lesser extent, in skeletal muscle, kidney and heart cells.

Causes of raised plasma ALT activities

1. Acute viral or toxic hepatitis
2. Circulatory failure with ‘shock’ and hypoxia

The ALT is **more specific** for hepatic disease because AST may be present also in skeletal muscle and other tissues. AST is **more sensitive** than ALT.

2. Lactate dehydrogenase (LDH)

Lactate dehydrogenase catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes; measurement of plasma total LDH activity is therefore a non-specific marker of cell damage.

Causes of raised plasma total LDH activity

The LDH activity increased in myocardial infarction, some haematological disorders (diseases such as megaloblastic anaemia, acute leukaemias and lymphomas), viral hepatitis and skeletal muscle disease.

Five main isoenzymes can be detected by electrophoresis and are referred to as LDH_1 to LDH_5 {LDH_1 (HHHH), LDH_2 (HHHM), LDH_3 (HHMM), LDH_4 (HMMM) and LDH_5 (MMMM)}. LDH_1, predominates in cells of cardiac muscle, erythrocytes and kidney. The LDH_2 and LDH_3 occurs in acute leukaemia. The LDH_5, is the most abundant form in the liver and in skeletal muscle.

3. Creatine kinase (CK)

Creatine kinase is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle.

Causes of raised plasma CK activities

1. Physiological: neonatal period, plasma CK is generally higher in Africans than in Caucasians

2. The CK increased in myocardial infarction, muscular dystrophies, rhabdomyolysis (the breakdown of skeletal muscle), after an intramuscular injection.

Creatine kinase consists of two protein subunits, M and B, which combine to form three isoenzymes, BB (CK-1), MB (CK-2) and MM (CK-3). The CK-MM (CK-3) is the predominant

isoenzyme in skeletal and cardiac muscle. The CK-MB (CK-2) accounts for about 35 per cent of the total CK activity in cardiac muscle and less than 5 per cent in skeletal muscle; its plasma activity is always high. The CK-BB(CK-1) is present in high concentrations in the brain.

4. Amylase

Amylase breaks down starch and glycogen to maltose. It is present at a high concentration in pancreatic juice and in saliva. 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-abdominal and extra-abdominal conditions that cause similar acute abdominal pain; thus a high result is **not a specific** diagnostic marker for acute pancreatitis.

5. Lipase

Sometimes, when it is difficult to interpret plasma amylase results, it may be more useful to measure plasma lipase. This enzyme is also derived from the pancreas but is **more specific** for pancreatic pathology. In addition, lipase has a **longer half-life** than amylase and therefore may be more useful in the diagnosis of **acute pancreatitis**.

6. Alkaline phosphatase (ALP)

The ALPs are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in most tissues but are in particularly high concentration in bones, liver, intestine, placenta and kidney.

Causes of raised plasma ALP activities

A. Physiological causes for raised serum ALP

1. **Pregnancy:** placental ALP appears in serum of pregnant women only during last trimester of pregnancy
2. **In infancy** due to predominance of bone ALP isoenzyme
3. **In children**, the total activity increases during the bone growth spurt
4. **In the elderly**, the plasma bone isoenzyme activity may increase slightly

B. Pathological causes for raised serum ALP

1. **Bone disease:** rickets and osteomalacia, Paget's disease of bone (may be very high) and bone malignancy
2. **Liver disease:** cholestasis, tumours and granulomas
3. **Inflammatory bowel disease:** the gut ALP isoenzyme can be increased in ulcerative colitis.

7. Acid phosphatase (ACP)

The ACP is found in cells of the prostate, liver, erythrocytes, platelets and bone. The main indication for estimation was to help diagnose **prostatic carcinoma** and to monitor its treatment. Estimation has been largely replaced by the measurement of plasma prostate-specific antigen (PSA), a protein derived from the prostate. This test is **more specific and sensitive** for diagnosis and monitoring treatment and has essentially rendered the plasma ACP assay obsolete in the diagnosis and management of prostatic carcinoma because:

a. Hemolyzed blood samples should also be avoided, as ACP is found in erythrocytes
b. Normally, the ACP drains from the prostate, through the prostatic ducts and into the urethra and very little can be detected in plasma
c. In extensive prostatic carcinoma, particularly if it has spread extensively or has metastasized, plasma ACP activity rises, probably because of the increased number of prostatic ACP-containing cells
d. Another problem with plasma ACP is that its concentration can increase after rectal examination