Clinical enzymology

Dr. mohammed talat

- Enzymology can be defined as the assay of an enzyme(s) in body fluids, usually blood, that can be used diagnostically or to monitor a clinical condition.

- An enzyme is a protein that catalyses one or more specific biochemical reactions. It is usually easier to measure enzyme *activity* in body fluids, by monitoring changes in either substrate or product concentrations, than to measure enzyme protein concentration directly, although this is sometimes done.

- However, measurement of the enzyme protein concentration is more specific and less prone to analytical variation.

- Generally, enzymes are present in cells at much higher concentrations than in plasma. Some occur predominantly in cells of certain tissues, where they may be located in different cellular compartments such as the cytoplasm or the mitochondria.

- Normal’ plasma enzyme concentrations reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation.

- The enzyme activity in plasma may be:
- *higher than normal*, due to the proliferation of cells, an increase in the rate of cell turnover or damage or in enzyme synthesis (induction), or to reduced clearance from plasma,

- *lower than normal*, due to reduced synthesis, congenital deficiency or the presence of inherited variants of relatively low biological activity –examples of the latter are the cholinesterase variants.

- Sometimes macroenzymes are found, that is to say, a high-molecular-weight form of a native enzyme. Often these are enzymes [such as lactate dehydrogenase (LDH), creatine kinase (CK) and alkaline phosphatase(ALP)] complexed with immunoglobulins and are more common in individuals with autoimmune disease. It is important to recognize macroenzymes as they can sometimes cause diagnostic confusion

- changes in plasma enzyme activities may be useful to detect and localize tissue cell damage or proliferation, or to monitor the treatment and progress of disease.

ASSESSMENT OF CELL DAMAGE AND PROLIFERATION

Plasma enzyme levels depend on the extent of cell damage and the rate of release from damaged cells, which, in turn, depends on the rate at which damage is occurring. In the absence of cell damage, the rate of release depends on the degree of induction of enzyme synthesis and the rate of cell proliferation.

- These factors are balanced by the rate of enzyme clearance from the circulation.
- Acute cell damage, for example in viral hepatitis, may cause very high plasma aminotransferase activities that reduce as the condition resolves.

- By contrast, the liver may be much more extensively involved in advanced cirrhosis but the *rate* of cell damage is often low, and consequently plasma enzyme activities may be only slightly raised or within the reference range. In very severe liver disease, plasma enzyme activities may even fall terminally when the number of hepatocytes is grossly reduced

- Relatively small enzymes, such as amylase, can be cleared by the kidneys. Thus, plasma amylase activity may be high as a result of renal glomerular impairment rather than pancreatic damage. However, most enzymes are large proteins and may be catabolized by plasma proteases before being taken up by the reticuloendothelial system.

- In healthy individuals, each enzyme has a fairly constant and characteristic biological half-life, a fact that may be used to assess the time since the onset of an acute illness. After a myocardial infarction, for example, plasma levels of CK and aspartate aminotransferase (AST) fall to normal before those of LDH, which has a longer half-life

Localization of damage

- Most of the enzymes commonly measured to assess tissue damage are present in nearly all body cells, although their relative concentrations in certain tissues may differ. Measurement of the plasma activity of an enzyme known to be in high concentration within cells of a particular tissue may indicate an abnormality of those cells, but the results will rarely enable a specific diagnosis to be made.

- For example, if there is circulatory failure after a cardiac arrest, very high plasma concentrations of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance.

- The distribution of enzymes within cells may differ. Alanine aminotransferase (ALT) and LDH are predominantly located in cytoplasm, and glutamate dehydrogenase (although this is not usually measured clinically) in mitochondria, whereas AST occurs in both these cellular compartments. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities

- The diagnostic precision of plasma enzyme analysis may be improved by the following:
- *Serial enzyme estimations* The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or, occasionally, impaired clearance.

- *Isoenzyme determination* Some enzymes exist in more than one form; these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues, such identification will give more information than the measurement of plasma total enzyme activity;

- for example, CK may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium.

- *Estimation of more than one enzyme* Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For example, although both ALT and AST are abundant in the liver, the concentration of AST is much greater than that of ALT in heart muscle.

Non-specific causes of raised plasma enzyme activities.

Before attributing a change in plasma enzyme activity to a specific disease process, it is important to exclude the presence of factitious or non-specific causes.

- Slight rises in plasma ALT and AST activities are common, non-specific findings in many illnesses. Moderate exercise, or a large intramuscular injection, may lead to a rise in plasma CK activity; isoenzyme determination may identify skeletal muscle as the tissue of origin.

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

- Analytical factors
- The total concentration of all plasma enzyme proteins is less than 1 g/L. The results of enzyme assays are not usually expressed as concentrations, but as activities.

- Changes in concentration may give rise to proportional changes in catalytic activity, but the results of such measurements depend on many analytical factors,
- including:
- substrate concentration,
- product concentration,
- enzyme concentration,
- reaction temperature,

- reaction pH,
- presence of activators or inhibitors.

- Non-disease factors
- Examples of non-disease factors affecting enzyme activities include the following.

- 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 and peaks during the pubertal bone growth spurt before falling to adult levels. A second peak occurs in the elderly.

- Sex
- Plasma GGT activity is higher in men than in women. Plasma CK activity is also higher in males, probably in part due to their increased muscle bulk.
- Race/ethnicity
- Plasma CK activity is higher in black people and Afro-Caribbeans than in white people.

- Physiological conditions
- Plasma ALP activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme. Several enzymes, such as AST and CK, rise moderately in plasma during and immediately after labour or strenuous exercise.

Aminotransferases

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. The aminotransferases are used as part of the biochemical liver profile.

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

- *Moderate to slight increase* (usually less than five
- times URL):
- – Hepatic steatosis [fatty liver or non-alcoholic
- fatty liver disease (NAFLD)],
- – cirrhosis (may be normal sometimes),
- – infectious mononucleosis (due to liver
- involvement),
- – cholestatic jaundice,
- – malignant infi ltration of the liver (may be
- normal),

NORMAL PLASMA ENZYME ACTIVITIES

- *Causes of raised plasma aspartate aminotransferase activities*
- *Artefactual*: due to in vitro release from erythrocytes if there is haemolysis or if separation of plasma from cells is delayed.
- *Physiological*: during the neonatal period (about 1.5 times the upper adult reference limit).
- *Marked increase* (may be greater than 5–10 times the
- upper reference limit or URL):
- – circulatory failure with ‘shock’ and hypoxia,
- – myocardial infarction,
- – acute viral or toxic hepatitis.

- – skeletal muscle disease,
- – after trauma or surgery (especially after cardiac
- surgery),
- – severe haemolytic episodes (of erythrocyte
- origin),
- – certain drugs.
- Note that AST is not specific for hepatic disease.

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

- *Causes of raised plasma alanine aminotransferase activities*
- *Marked increase* (may be greater than 5–10 times
- URL):
- – circulatory failure with ‘shock’ and hypoxia,
- – acute viral or toxic hepatitis.
- *Moderate to slight increase* (usually less than five
- times URL):
- – Hepatic steatosis (fatty liver or NAFLD),
- – cirrhosis (may be normal sometimes),
- – infectious mononucleosis (due to liver
- involvement),
- – liver congestion secondary to congestive
- cardiac failure,
- – cholestatic jaundice,

- coeliac disease,
- – surgery or extensive trauma and skeletal muscle
- disease (much less affected than AST),
- – certain drugs.
- Note that ALT is more specifi c for hepatic disease than AST.
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- Lactate dehydrogenase
- 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 lactate dehydrogenase activity

- *Artefactual*: due to in vitro haemolysis or delayed separation of plasma from whole blood.
- *Marked increase* (may be greater than 5–10 times
- URL):
- – circulatory failure with ‘shock’ and hypoxia,
- – myocardial infarction

- some haematological disorders: in blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas, very high levels (up to 20 times the URL in adults) may be found. In cases of lymphoma LDH can be used as a tumour marker.

- Smaller increases occur in other disorders of erythropoiesis, such as thalassaemia, myelofi brosis and haemolytic anaemias, renal infarction or, occasionally, during rejection of a renal transplant.

- *Moderate to slight increase* (usually less than five
- times URL):
- – viral hepatitis,
- – malignancy of any tissue,
- – skeletal muscle disease,
- – pulmonary embolism,
- – infectious mononucleosis,
- – certain drugs.

- Isoenzymes of lactate dehydrogenase
- Five main isoenzymes can be detected by electrophoresis and are referred to as LDH1 to LDH5. LDH1, the fraction that migrates fastest towards the anode, predominates in cells of cardiac muscle, erythrocytes and kidney. The slowest moving isoenzyme, LDH5, is the most abundant form in the liver and in skeletal muscle.

- Whereas in many conditions there is an increase
- in all fractions, the finding of certain patterns is of
- diagnostic value:
- Predominant elevation of LDH1 and LDH2 (LDH1 more than LDH2) occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction. Predominant elevation of LDH2 and LDH3 occurs in acute leukaemia; LDH3 is the main isoenzyme elevated as a result of malignancy of many tissues. Elevation of LDH5 occurs after damage to the liver or skeletal muscle.

- Creatine kinase
- 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.

- Isoenzymes of creatine kinase
- 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).
- *CK-MM* is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.

- *CK-MB* 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 after myocardial infarction

- *CK-BB* is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts.

- Causes of raised plasma creatine kinase activities
- *Artefactual*: due to in vitro haemolysis, using most
- methods.
- *Physiological*:
- – neonatal period (slightly raised above the adult
- URL),
- – during and for a few days after parturition,
- – plasma CK is generally higher in Africans than
- in Caucasians.

- *Marked increase* (may be greater than 5–10 times
- URL):
- – dermatomyositis and polymyositis,
- – ‘shock’ and circulatory failure,
- – myocardial infarction,
- – muscular dystrophies,
- – rhabdomyolysis (the breakdown of skeletal
- muscle),
- – necrotizing fasciitis.

- *Moderate to slight increase* (usually less than five
- times URL):
- – muscle injury,
- – infections, for example viral,
- – after surgery (for about a week),
- – physical exertion – there may be a significant
- rise in plasma activity after only moderate
- exercise, muscle cramp or following an
- epileptic fit,

- after an intramuscular injection,
- – hypothyroidism
- – alcoholism (possibly partly due to alcoholic
- myositis),– some cases of cerebrovascular accident and
- head injury,

- malignant hyperpyrexia,
- – certain drugs, for example statins, ciclosporin,
- cocaine,
- – glycogen storage diseases,
- – carnitine palmityl transferase defi ciency.

- Aldolase
- This glycolytic enzyme has been measured in plasma as a marker of muscle disease. However, generally it offers no major advantage over CK and is now rarely used.

- Amylase
- Amylase (molecular weight 45 kDa) breaks down starch and glycogen to maltose. It is present at a high concentration in pancreatic juice and in saliva and may be extracted from other tissues, such as the gonads, Fallopian tubes, skeletal muscle and adipose tissue. Being of relatively low molecular weight, it is excreted in the urine.

- 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 late-presenting acute pancreatitis.

- Alkaline phosphatase
- 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 the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta

- Thank you