Plasma Enzymes in Diagnosis

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Most 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 levels reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation.

Plasma contains several enzymes, some of which are functional in the plasma, while others are merely present in plasma due to leakage from tissues. Lipoprotein lipase, pseudocholinesterase and enzymes concerned in the coagulation of blood and the dissolution of the blood clot are enzymes that serve a function in the plasma. Though many other enzymes have no function in the plasma, they are still useful as diagnostic tools. Measurement of their levels in plasma offers valuable information about diseases involving the tissue of their origin.

It is easier to measure enzyme activity in body fluids, by monitoring changes in either substrate or product concentrations, than to measure enzyme protein concentration directly.

The enzyme activity in plasma may be:

- Increased due to 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, very occasionally due to reduced synthesis or congenital deficiency.
Changes in plasma enzyme activities may sometimes help to detect and localize tissue cell damage or proliferation, or to monitor treatment and progress of disease.

**Assessment of Cell Damage and Proliferation**

Plasma enzyme levels depend on:

1. The rate of release from damaged cells which, in turn, depends on the rate which damage is occurring;
2. The extent of cell damage.

In the absence of cell damage the rate release depends on:

1. The rate of cell proliferation:
2. The degree of induction of enzyme synthesis

These factors are balanced by:

- The rate of enzyme clearance from circulation.

Acute cell damage, for example in viral hepatitis, may cause very high plasma enzyme activities that fall 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 be within the reference range. In very severe liver disease plasma enzyme activities may even fall terminally, when the number of hepatocytes is grossly reduced.

It is not known how most enzymes are removed from, or their action inhibited in the circulation. Relatively small peptides, such as α-amylase, can be cleared by the kidneys: most enzymes are large proteins and are probably catabolized by plasma proteases before being taken up by the reticuloendothelial system. In health each enzyme has a fairly constant and characteristic biological half-life; knowledge of this half-life may be of help in assessing the time since the onset of an acute illness. After a myocardial infarction, for example, plasma levels of creatine kinase and aspartate transaminase fall to normal before those of lactate dehydrogenas, which has a longer half-life. The half-life may be lengthened if there is circulatory impairment. Renal glomerular impairment may delay the rate of fall of those plasma enzymes cleared through the kidneys. For example plasma amylase activity may be high due to renal glomerular impairment, rather pancreatic damage.
**Localization of Damage**

Most of the enzymes commonly measured to assess tissue damage are present in nearly all 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 levels of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance: the raised plasma levels of 'cardiac' enzymes do not necessarily mean that a myocardial infarct caused the arrest.

The diagnostic precision of plasma enzyme analysis may be improved by:

1. **Estimation of more than one enzyme.** Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For instance, although both alanine and aspartate transaminases are abundant in the liver, the concentration of aspartate transaminase is much greater than that of alanine transaminase in heart muscle.

2. **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, creatine kinase may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium.

3. **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 of impaired clearance.

The distribution of enzymes within cells may differ. Alanine transaminase and lactate dehydrogenase are predominantly located in cytoplasm and glutamate dehydrogenase in mitochondria, whereas aspartate transaminase 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.
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 nonspecific causes.

Slight rises in plasma aspartate transaminase activities are common, non-specific findings in many illnesses. Moderate exercise, or a large intramuscular injection, may lead to a rise in plasma creatine kinase activity; isoenzyme determination may identity skeletal muscle as the tissue of origin.

Some drugs, may induce synthesis of the microsomal enzyme, gamma-glutamyltransferase, 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 disease this may occur if for example the plasma enzyme forms.

- macromolecules (aggregates), such as in macroamylasemia.
- complexes with immunoglobulins. as occasionally occur with lactate dehydrogenase, alkaline phosphatase, creatine kinase.

Factors Affecting Results of Plasma Enzyme Assays

1. Analytical factors affecting results. The total concentration of all plasma enzyme proteins is less than 1 g/L. 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. These include the concentrations of the substrate and product, the pH and temperature at which the reaction is carried out. The type of buffer, and the presence of activators or inhibitors. Because the definition of 'international units' does not take these factor into account, results from different laboratories, apparently expressed in the same units, may not be directly comparable. Therefore, plasma enzyme activities must be interpreted in relation to the reference ranges from the issuing laboratory.
2. Physiological factors affecting enzyme activities include for example:
   a. Age: plasma aspartate transaminase activity is moderately higher during the neonatal period than in adults: plasma alkaline phosphatase activity of bony origin is higher in children than in adults and peaks during the pubertal bone growth spurt before falling to adult levels.
   b. Sex: plasma γ-glutamyltransferase activity is higher in men than in women.
   c. Physiological conditions:
      Plasma alkaline phosphatase activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme: several enzymes, such as the transaminases and creatine kinase rise moderately in plasma during and immediately after labour or strenuous exercise.
      Plasma enzyme activities must be interpreted in relation to the sex and age-matched reference ranges of the issuing laboratory.
Genetic basis for enzyme synthesis:

Since all enzymes are proteins, their synthesis follows the general pattern of protein synthesis and is regulated by genes. For every enzyme, there is said to be one gene (one gene, one enzyme hypothesis); where an enzyme is a complex protein containing more than one protein subunit more than one gene may be concerned in its synthesis. Genetic mutation results in abnormal DNA code and synthesis of an abnormal enzyme protein. Since the abnormal enzyme cannot serve the normal function, a metabolic abnormality occurs and this is transmitted to the progeny. Such transmittable abnormalities of metabolism due to abnormal enzyme molecules are known as: 'Molecular diseases' or 'Inborn Errors of Metabolism'. Phenylketonuria, alkaptonuria, pentosuria, glycogen storage disease, galactosemia and cystinuria are but a few of several known molecular diseases.

1. Phenylketonuria: is an autosomal recessive disorder caused by an abnormality of the phenylalanine hydroxylase. Because phenyl alanine cannot be converted to tyrosine it accumulates in plasma and is secreted in the urine with its metabolites, such as phenylpyrovaleric acid; the disease acquired its name from the detection of the latter "phenylketone" in the urine.(see diagram below)

Diagram showing the metabolism of tyrosine and some inborn errors of the aromatic amino acid pathways. Substances highlighted may be present in abnormal amounts in certain inborn errors of metabolism.

1 phenylalanine hydroxylase phenylketonuria
2 homogentisic acid oxidase alkaptonuria
3 tyrosinase albinism
4 thyroid enzymes thyroid dyshormonogenesis

Phenylalanine → phenylpyruvic (phenylketone) etc.

Phenylalanine

Melanin

Adrenaline

Homogentistic acid

TCA cycle

Diagnosis: Genetica basis for enzyme synthesis:
2. Alkaptonuria: is an autosomal recessive disorder due to the deficiency of homogentisic acid oxidase. Homogentisic acid accumulates in tissue and blood and is passed in the urine. Oxidation and polymerization of homogentisic acid produces the pigment alkapton in much the same way as polymerization of DOPA produce melanin. Deposition of alkapton in cartilages, with consequent darkening is called ochronosis and result in visible darkening of the cartilages of the ears.. Conversion of homogentisic acid to alkapton is accelerated in alkaline conditions and most obvious abnormality in alkaptonuria is darkening of the urine as it becomes more alkaline on standing. The condition is compatible with normal life span despite the tendency for patient to develop arthritis in later life. (refer to previous diagram)

3. glycogen storage disease: a deficiency of one of the enzymes involved in the glycogenesis or glycogenolysis results in the accumulation of normal or abnormal glycogen with hepatomegaly: in von Gierk's disease, the least rare glycogen storage disorder, there is a deficiency of glucose-6-phosphate. Fasting hypoglycemia occurs because the enzyme is essential for the conversion of glucose-6-phosphate to glucose.

4. galactosaeemia an autosomal recessive disorder due to a deficiency of galactose-1-phosphate uridylytransferase , may cause cirrhosis of the liver if untreated.

5. cystinuria: is an autosomal recessive inherited abnormality of tubular reabsorption, with excessive urinary excretion of the dibasic amino acids: cystine, arginine and lysine.