

CLINICAL BIOCHEMISTRY

Clinical enzymology and its applications

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11-Jan-2007

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Key words

Clinical enzymology; Enzyme classification; Structure; Activation energy; Factors affecting enzyme activity; Inhibition of enzyme activity; Enzymes and isoenzymes; Functional and nonfunctional plasma enzymes; Measurement of enzyme activity; Aspartate transaminase (AST) / Alanine transaminase (ALT); Creatine kinase; LDH; Alkaline phosphatase; Acid phosphatase; GGT; Amylase; Lipase; Cholinesterase; 5'NT; Gastric function test; Renal function test; Liver function test

Clinical Enzymology

Clinical enzymology can be described as the branch of science which deals with the application of enzyme analysis to the diagnosis and treatment of disease. Enzymes are proteins which have catalytic properties and activate substrates. They are the catalysts of all biological and metabolic reactions in body. The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance. This is because the presence of these enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood. Hence, when a physician indicates that he/she is going to assay for liver enzymes, the purpose is to ascertain the potential for liver cell damage.

Historical relevance of clinical enzymology

This diagnostic relevance of enzymes was put into practice as early as the 1900s. One of the earliest reported enzyme measurement in body fluids was that of amylase in urine by Wohlgemuth in 1908. The use of serum as the diagnostic fluid for measuring enzyme activity started in 1920s and 1930s with studies on alkaline phosphatase (ALP) in bone and liver disease conducted by Kay, King, Bodansky and Roberts. The relevance of alterations in acid phosphatases (ACP) activity was first appreciated by Kutscher and Wolbergs; and Gutman and Gutman in prostate cancer. The modern diagnostic enzymology with better sensitivity of detection and analytical methods took shape in the 1940s and 1950s. Warburg and Christian observed an increased activity of glycolytic enzymes in sera of tumor-bearing mice and later, Wroblewski and Karmen reported a transitory rise in the levels of glutamic-oxaloacetic transaminase activity (also known as aspartate aminotransferase) in serum after acute myocardial infarction.

Enzymes nomenclature, classification, structure and specificity

Enzymes are globular proteins. Their folded conformation creates an area known as the active site. The nature and arrangement of amino acids in the active site of the enzyme make it specific for only one type of substrate. They enhance the rates of the reaction by many folds, approximately 10^6 times faster than the corresponding noncatalyzed reaction. So enzymes are highly efficient as well as extremely selective for the type of reaction they catalyze.

Enzymes can be classified on the basis of the type of reaction it catalyzes (International Union of Biochemists [IUB] classes). They are:

1. Oxidoreductases: Involved in oxidation and reduction of substrates.
2. Transferase: Help in transfer of a particular group such as methyl or glycosyl groups from one substrate to another.
3. Hydrolases: Bring about hydrolytic cleavage of bonds like C-C, C-O, C-N, etc.
4. Lyases: Facilitate removal of small molecule from a large substrate leaving double bonds; also add groups to double bonds.
5. Isomerases: Isomerisation of substrate.
6. Ligases: Involved in joining together of two substrates, coupled to the hydrolysis of an ATP.

Unit of enzyme activity

The amount of enzyme in a sample is measured by the rate of reaction catalyzed by the enzyme. This rate is directly proportioned to the amount of enzyme and is expressed in enzyme unit, IU/L. Isoenzyme Physically distinct and separable form of the given enzyme present in different cell types. It is of diagnostic value. Can be separated by electrophoresis e.g. isoenzymes of lactate dehydrogenase are LD1, LD2, LD3, LD4 and LD5

Principle behind diagnosing diseases with enzymes

The basic principle of using the enzyme levels for diagnosing disease is based on comparing the changes in activity in serum or plasma of enzymes which are predominantly present intracellularly and are secreted in the serum in very low active amounts. A sensitive analysis would give insight into the pathological changes and nature of the disease. The large number of enzymes present in the human body are hence mostly intracellular, and those which are secreted into the blood can be subsequently divided into two classes; 1) Plasma specific enzymes which include serine protease procoagulants such as thrombin, factor XII, Factor X and others. These are known as functional plasma enzymes. 2) Secreted enzymes: Lipases present in salivary glands, gastric oxyntic glands and pancreas; α -amylase in salivary glands and pancreas, lactate dehydrogenase enzyme and alkaline phosphatase etc. These are known as non-functional plasma enzymes as they have no function as such in the blood but are present in the circulation because of the normal wear and tear processes of the cells. As the enzymes and their isoforms may belong to varied tissue types, it is of significant relevance to have a detailed knowledge of isoenzymes of the enzymes under study and their enzymatic properties like kinetics, effect of factors like temperature and pH, rate of release from the cells of origin and rate of clearance from circulation.

How enzymes work

For two molecules to react they must collide with one another. They must collide in the right direction (orientation) and with sufficient energy. Sufficient energy means that between them they have enough energy to overcome the energy barrier to reaction. This is called the **activation energy**. Enzymes have an **active site**. This is part of the molecule that has just the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the **substrate**. An enzyme-catalysed reaction takes a different 'route'. The enzyme and substrate form a reaction intermediate. Its formation has lower activation energy than the reaction between reactants without a catalyst.

A simplified picture:

Route A reactant 1 + reactant 2 $\rightarrow$ product

Route B reactant 1 + enzyme $\rightarrow$ intermediate

intermediate + reactant 2 $\rightarrow$ product + enzyme

So the enzyme is used to form a reaction intermediate, but when this reacts with another reactant the enzyme reforms.

Factors affecting enzyme activity

Enzymes are very efficient catalysts for biochemical reactions. They speed up reactions by providing an alternative reaction pathway of lower activation energy.

Like all catalysts, enzymes take part in the reaction - that is how they provide an alternative reaction pathway. But they do not undergo permanent changes and so remain unchanged at the end of the reaction. They can only alter the rate of reaction, not the position of the equilibrium.

Most chemical catalysts catalyze a wide range of reactions. They are not usually very selective. In contrast enzymes are usually highly selective, catalyzing specific reactions only. This specificity is due to the shapes of the enzyme molecules.

Many enzymes contain small non-protein molecules and metal ions (called the prosthetic groups, cofactors and coenzymes) that participate directly in substrate binding or catalysis along with the protein part of the enzymes. Prosthetic groups are the organic groups that are tightly bound to the enzymes structure by covalent or noncovalent forces. Cofactors associate with enzymes or substrate reversibly during the enzyme-substrate interactions. Prosthetic groups and Cofactors facilitate the binding of substrates to the enzymes by making them more electrophilic and nucleophilic. Coenzymes serve as a group transfer reagents, they help in transfer of the substrate from their point of generation to the point of utilization. The proteins in enzymes are usually globular. The intra- and inter-molecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature and pH. This affects shapes and so the catalytic activity of an enzyme is pH and temperature sensitive.

Temperature

As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 °C) for the enzymes in human cells.

Above this temperature the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

pH

Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.

Concentration of enzyme and substrate

The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (Fig. 1).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate

complex has to dissociate before the active sites are free to accommodate more substrate. Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration.

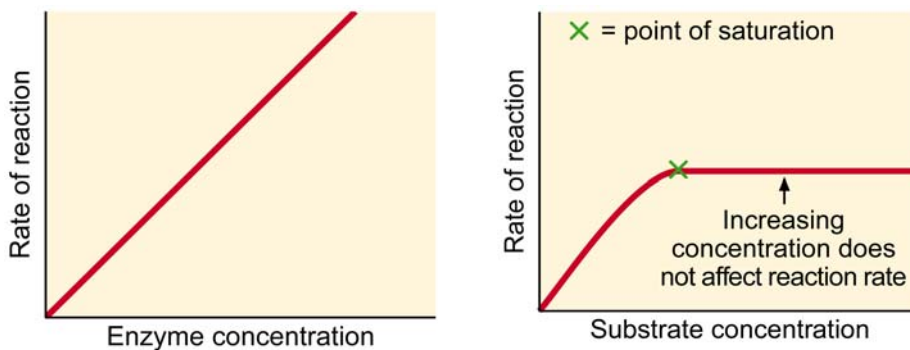


Fig. 1: Effect of the concentrations of enzyme and substrate on the rate of an enzyme-catalyzed reaction

Inhibition of enzyme activity

Some substances reduce or even stop the catalytic activity of enzymes in biochemical reactions. They block or distort the active site. These chemicals are called inhibitors, because they inhibit reaction.

Inhibitors that occupy the active site and prevent a substrate molecule from binding to the enzyme are said to be **active site-directed** (or **competitive**, as they 'compete' with the substrate for the active site).

Inhibitors that attach to other parts of the enzyme molecule, perhaps distorting its shape, are said to be **non-active site-directed** (or **non competitive**).

Enzymes, isoenzymes and their relevance in diagnosis

Enzymes are 'protein catalysts' and can be best described as substances that increase the rate of a particular chemical reaction without being permanently consumed or altered in any reaction. Enzymes are required for all biological reactions occurring in the body. As a catalyst they are required in amounts much less than the substrate they act on. And it is this catalytic property which makes enzymes a very sensitive indicator of any biochemical or pathological change occurring in the body. An increase in the enzymatic levels in the blood compared to their normal levels is a good indicator of altered patterns.

Enzymes exist in multiple forms in the human body. These multiple molecular forms of the enzymes are referred to as the **isoenzymes** or **isozymes**. The various forms of an enzyme can be distinguished from each other on the basis of differences in various physical properties, such as electrophoretic mobility or resistance to chemical or thermal inactivation. They often also show quantitative differences in their catalytic properties. Such enzyme variants are found sometimes within a single organ or even within a single cell-type. However, all isoenzymes retain the ability to catalyze the basic reaction unique to that particular enzyme.

Factors leading to isoenzymes formation

Isoenzymes may be formed as a result of either genetic variations or non-genetic causes.

Genetic origin of isoenzymes

Isoenzymes which are formed due to existence of more than one gene locus coding for the protein catalyst are the true isoenzymes. The genes at different loci have over the course of time evolved such that their protein structure might not be identical, however, they are recognizably similar ei they are isoenzymes. It is also not necessary that the multiple genes responsible for a particular set of isoenzymes, should necessarily be present on the same chromosome. For example the human pancreatic and salivary amylase are located on the same chromosome 1, however, the malate dehydrogenase has mitochondrial and cytoplasmic isoenzymes, the coding genes of which are located separately on chromosome 7 and 2 respectively.

Enzymes also often exist in different molecular forms and vary from one individual to another. Allelic genes are alternative forms of a gene that occur at a particular locus and give rise to gene products with the same function. The isoenzymes which result from allelic genes are called allelozymes. For example more than 150 forms of Glucose-6-phosphate dehydrogenase of human erythrocytes have been identified, each form being identified by a different allele at the locus of the gene that codes for the enzyme on the X chromosome. Such alleles might be extremely rare whereas others may exist in a sizable number in a population. When isoenzymes due to variation at a single locus occur with appreciable frequency in a population, the population is said to be polymorphic with respect to the isoenzyme in question.

Isoenzymes may also be formed in case of oligomeric enzymes ei molecules made up of various subunits. The association of different types of subunits in various combination gives rise to a range of active enzyme molecules. In such cases the the subunits may be derived from different structural genes, originating from either multiple loci or alleles. Such hybrid molecules also recognized as isoenzymes and are known as hybrid isoenzymes.

With such a wide category of isoenzymes, it is important to understand that true isoenzymes are multiple forms of an enzyme which possess the ability to catalyze the characteristic reaction of the enzyme but may differ in structure as they are coded by distinct structural genes.

Although by definition only the above mentioned categories should be considered as isoenzyme, however, the term isoenzyme is still used to define any multiple form of an enzyme.

Non genetic causes of multiple forms of an enzyme

Multiple forms of an enzyme commonly known as isoforms of the enzyme may arise due to various types of post-translational modification of the enzyme molecule

Regulation of enzyme levels in serum and plasma

The balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood determines the level of activity of the enzyme. There are two crucial factors which determine the rate of entry of enzymes into the circulation from the cells of origin. The first being those that affect the rate of leak from the cells and the second are those that actually reflect altered rates of enzyme production, due to either increased synthesis of the enzyme in response to metabolic alterations in the cell or due to increased proliferation of the cell itself.

How enzymes leak from cells

Enzymes are essentially harbored inside cells of their origin and restrained within the plasma membrane. As long as the integrity of the plasma membrane is maintained the enzymes do not leak out of the cell. This integrity is maintained by the cell's ATP production. ATP production of the cell can be hampered in many ways, such as the loss of oxygen carrying capacity and blood supply; treatment with chemicals and drugs and other environmental pollutants, extreme physical stress such as heat, radiation; exposure to microbial agents and subsequent infection; disruption or malfunction of the immune system; genetic defects leading to metabolic disorders and nutritional disorders. One or many of such distress cause the plasma membrane to deteriorate. During the early stages of this loss of integrity there is an efflux of potassium ions and an increased influx of sodium ions. This leads to increased water retention in the cell leading to swelling. In later stages, calcium influx occurs which acts as a stimulus to the intracellular enzymes leading to their hyperactivity and an increase in cell damage and disruption of the cell membrane. Finally, all these processes lead to an increased production of free radical and oxidative damage and the membrane become leaky and molecules of all sizes eventually leak out depending on the extent of damage. The rate at which these enzymes leak out of the cell also depends on the sub-cellular location of the enzymes. The mitochondrial enzymes and others which are bound to the membranes of sub-cellular structures are not readily released into the circulations. Sensitive detection of such enzymes gives information to distinguish between damage only to the cell membrane from that of a necrotic damage. There is also a distinct variation of enzyme release with respect to the type of tissue damaged. For example, in case of myocardial infarction, the short episode of an attack leads to a rapid release of myocardial enzymes and in about 24 hours the enzyme levels resemble those present in the myocardial tissue. However, in chronic diseases such as those of liver, the enzyme release may continue over a prolonged period of time and may show a varied pattern depending on the variation in clearance of the enzyme and its leakage at various stages of the disease.

Alteration in enzyme production

A basal level of intracellular enzymes normally present in the plasma may be a result of wear and tear of cells or overflow of enzyme from healthy cells. A decrease in the levels may arise due to genetic deficiency such as in the case of ALP in hypophosphatasia or in case of diseased condition as in the case of decreased production of serum cholinesterase in liver disease.

In diagnosis of disease, an increase of enzyme production is often more appreciated. However, the levels of enzymes and their isoenzymes often varies during a normal growth of an individual. The various isoenzymes of ALP provide us with a good example of such

variations. For example bone Alkaline phosphatase (bALP) is found to be increased in growing children and such increase in the bALP is also observed during an increased osteoblastic activity in bone diseases. Similarly, placental ALP (pLAP) production starts towards the end of a normal pregnancy. An increase in ALP production is seen by the liver during biliary obstruction.

In prostate cancer, the acid phosphatase (ACP) levels increases due to the proliferating ACP-producing cells. However, this initial burst of ACP production may also decline if the cells metastasize far from the prostate and the fast changing metastatic cells become more and more unlike the cell of origin and lose their ACP producing capability. On the other hand a cancerous prostatic cell produces almost 10 times more amount of prostate specific antigen (PSA) as compared to its normal counterpart and would serve as a good marker for prostate cancer.

Clearance rate of enzymes

It has now become increasingly evident that most of the enzymes are removed from the circulation using the mechanism of receptor mediated endocytosis via the reticuloendothelial system largely involving bone marrow, spleen and the Kupffer cells of the liver and to some extent by nearly all other cells in the body. Receptor mediated endocytosis involves recognition and uptake of molecules, in this case, enzymes (proteins), by the cell surface receptors, followed by their fusion with the lysosomes, digestion of the proteins and eventual recycling of the receptors to the surface.

Since the enzymes are comparatively larger molecules, with the exception of amylases, the clearance from the glomerulus of the kidney is not physically feasible. Amylase is the only enzyme which is small enough to pass through the kidney. Amylase increase in conditions such as acute pancreatitis can hence be readily detected in the urine.

The half lives of enzymes in plasma varies from hours to days averaging 24-48 hours. When these enzymes complex with immunoglobulins, macroenzymes, the half life increases considerably. The rate of decay is expressed as in its k_d value which is the fractional disappearance rate and its half life by the equation: $k_d = 2.303 \log 2 / t_{1/2} = 0.693 / t_{1/2}$.

Specific enzymes and their isoenzymes are often recognized by their specific receptors of the surface of the cells of the reticuloendothelial system. The hepatic Kupffer cells endocytose enzymes derived from many tissue types such as the Lactate dehydrogenase 5 (LD-5), Creatine Kinase 3 (CK-3), adenylate kinase (AK), both the cytoplasmic and mitochondrial Aspartate aminotransferase or transaminase (AST), malate and alcohol dehydrogenase. The Kupffer cells are known to show affinity towards the lysine group present in all these enzymes.

The variation of clearance rate of enzymes and their isoforms varies under different pathological condition. For example, the intestinal ALP (iALP) is a glycoprotein with a galactosyl terminal group. This is recognized by a galactosyl-specific receptor on the hepatocyte membrane and undergoes endocytosis. Due to this specificity in recognition, the process is very rapid and in normal conditions the half life of iALP is very small. But in case of hepatic cirrhosis, the cell mass of the hepatocytic cells and consequently the receptors on its surface is considerable low, and this leads to an increase in the half life of the iALP and reduced clearance rate. The clearance rate of other isoforms of ALP is not the same as iALP.

The other ALP isoforms are sialoproteins are not recognized by the galactosyl terminal receptor and hence, their clearance rate from the blood is much higher. Many of these are now known to be excessively sialylated in malignant cells, again, prolonging their half lives. This example indicates the importance of knowing not only the pathological conditions of the disease but also the mechanism of enzyme clearance from plasma.

Selection of enzyme tests

There are number of factors which have to be kept in account while selecting the enzyme to measure in the serum for diagnostic or prognostic purposes. One of the important factors is the distribution of enzymes among various tissues. Another factor is that all intracellular enzymes cannot be equally valuable as an indicators of cellular damage even if they are specific and of high activity for particular tissue as they may get inactivated on entering into the blood stream after cellular damage, ie isocitrate dehydrogenase of heart muscles. So half life of the enzyme in the blood stream is also of significance, an enzyme with a very short half life in blood has little or no clinical value. Type of damage to a tissue can have significant effect eg mild inflammation to the cells will likely to increase the permeability of the cell membrane and allow only cytoplasmic enzymes to leak out into the blood, whereas in case of cell necrosis both cytoplasmic and mitochondrial enzymes will be detected in the blood. So knowledge of the intracellular distribution of enzymes can therefore assist in determining the nature and severity of a pathological process if suitable enzymes are assayed in the blood. Yet another factor is the availability of the assay and the feasibility of measuring the enzyme activity in the blood. The final diagnostic decisions are always made by assessing the results of the enzymes analyses along with the history of the patient's illness and the clinical findings.

Measurement of enzyme activity and chemical changes

Since the amount of active enzymes present in the blood sample is very low, measuring their catalytic activities which is directly proportional to the amount of active enzyme present in the sample is a very sensitive and specific method for the measurement of the enzymes present in the serum sample. Conversion of substrate into product in the presence of enzyme under optimum condition is followed, then either substrate consumed or product formed is measured in a defined time interval. There are two methods, fixed-time and continuous-monitoring methods. In fixed-time method the reaction is stopped at the end of a fixed interval of time. In continuous monitoring method the progress of the reaction is monitored continuously and enzyme activity is measured at intervals. In both the conditions substrate is always in excess for the reaction to progress, and enzyme is the only variable as limiting factor. So the rate of reaction depends only on enzyme concentration to be measured and is completely independent of substrate concentration. The reaction is said to follow zero-order kinetics as its rate is proportional to the zero power of the substrate concentration. As the reaction proceed, more and more substrate is consumed, the reaction rate declines and enter a phase of first order kinetics, dependent on substrate concentration. Enzyme assays are almost always made under conditions that are initially saturated with substrate concentration. The rate of reaction during the zero-order phase can be determined by measuring the product formed or substrate consumed during a fixed period of incubation, assuming that the rate has remained constant during this period. So the amount of product formed or substrate consumed ie the chemical changes during an enzyme catalyzed reaction is most commonly measured by photometric analysis. In the enzyme catalyzed reaction it may be accompanied by a change in the absorbance characteristics of some component of the assay system, either

in the visible or ultraviolet spectrum range eg determination of dehydrogenase activity by following change in absorbance at 340nm of the coenzyme NADH or NADPH during oxidation or reduction reaction.

Quality control

Much effort has been devoted to optimizing conditions for measuring the activities of enzymes of clinical importance. During measurement of an enzyme activity in a sample, optimum conditions, like optimum pH and temperature is to be maintained and various factors affecting the reaction rate are to be tightly controlled except for the concentration of active enzyme to be measured in the sample. There are large numbers of methods available for measuring the activity of a particular enzyme in clinical laboratories and different ways of interpreting the results. But national and international enzyme committees have recommended methods for clinical sample analyses that combine analytical reliability with applicability to current standards of instrumentation and skill in well-equipped laboratories. Control standards of known catalytic activity of particular enzymes to test are run along with the clinical test samples in the laboratory which help in the correct interpretation of the laboratory finding of the patient's sample. The application of quality control procedure is to ensure satisfactory analytical performance of enzyme assays various enzymes are available in lyophilized preparation from commercial sources and have a useful function in quality control. For all the available control samples, recalibration in the laboratory is necessary to take care of the laboratory to laboratory variations.

Measurement of isoenzymes and isoforms

Many enzymes in the body are determined by more than one structural gene locus and many are modified post translationally. The enzyme proteins coded by them are structurally not identical but are functionally similar. They are known as isoenzymes and isoforms respectively. Their distinctive properties in different organs helps in understanding organ-specific patterns of metabolism, whereas genetically determined variations in enzyme structure between individuals account for such characteristics as differences in metabolism that manifest themselves as hereditary metabolic diseases. From the diagnostic point of view, the existence of isoenzymes/isoforms provides opportunities to increase the diagnostic specificity and sensitivity enzyme assays carried out on blood samples. Isoenzymes/isoforms can be distinguished on the basis of differences in various physical properties such as electrophoretic mobility or resistance to chemical or thermal inactivation. Often there are significant quantitative differences in catalytic properties among them. Methods of isoenzymes/isoforms measurement depend on their structural and catalytic properties. They are assessed by their electrophoretic mobility, resistance to inactivation, response to inhibitors, and ratio of reaction with substrate analogs.

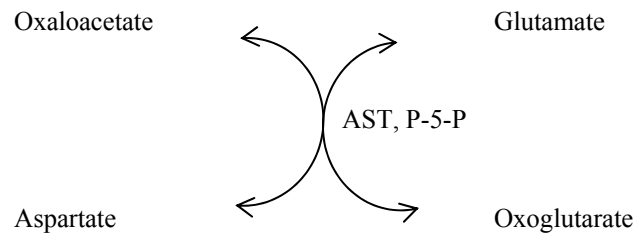
Some important enzymes in clinical practice

Estimation of enzymes activities in the serum has many applications in the diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased), assessing prognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before the occurrence of jaundice). Some important enzymes of clinical significances are discussed below:

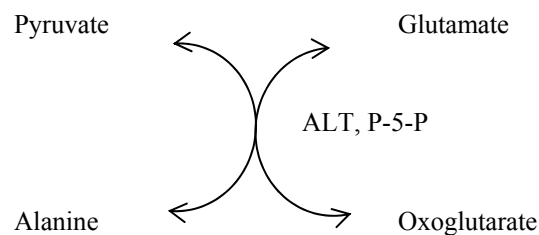
I. Aspartate transaminase (AST) and Alanine transaminase (ALT)

Transaminases are present in most of the tissues of the body. They catalyze the interconversions of the amino acids and 2-oxoacids by transfer of amino groups. Transaminases are specific for the amino acid from which the amino group has to be transferred to a keto acid. 2-oxoglutarate and glutamate couple serves as one amino group acceptor and donor pair in all amino transfer reactions.

AST catalyzes the interconversion of oxaloacetate to aspartate coupled with glutamate to oxoglutarate.



ALT catalyzes the interconversion of pyruvate to alanine coupled with glutamate to oxoglutarate.



The reactions catalyzed by AST and ALT are reversible but the equilibria of the reaction favor formation of aspartate and alanine respectively. In both the reactions pyridoxal-5'-phosphate functions as a prosthetic group in the amino transfer reactions. Normal serum values: AST (SGOT) - 0-41 IU/L and ALT (SGPT) - 0-45 IU/L. In newborns value up to 120 units for AST and 90 units for ALT is considered normal.

Clinical significance

The activities of both AST and ALT are high in tissues especially liver, heart, and muscles. Any damage or injury to the cells of these tissues may cause release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increase activities of these enzymes in the blood. Some increases in the activities of both the enzymes are seen after alcohol intake.

Liver diseases: Determinations of activities of AST and ALT in serum in patients with liver diseases like viral hepatitis and other forms of liver diseases with necrosis, give high values even before the appearance of clinical signs and symptoms like jaundice. Activity levels of 20 to 50 fold higher than normal are frequently seen in liver cells damage but it may reach as high as 100 times in severe damage to cells. Highest serum activities are seen between 7th and 12th days and return to normal levels by the 3rd to 5th week. In severe tissue damage ALT activity is higher than AST and the ALT:AST ratio becomes ≥ 1 (normally < 1). Some increase

in the activities of ALT and AST are seen in extrahepatic cholestasis. In cirrhosis the level of activities vary with the severity of the disease. It may increase only up to 5 fold of the normal activities. Up to 10 fold increase is seen in carcinoma of the liver. In both cirrhosis and carcinoma activity of AST is found to be higher than the ALT. Even though the activities of both AST and ALT are elevated in the serum of the patients with liver diseases, ALT is more liver specific enzyme as increased ALT activity in serum is hardly seen in tissues other than liver cell damage.

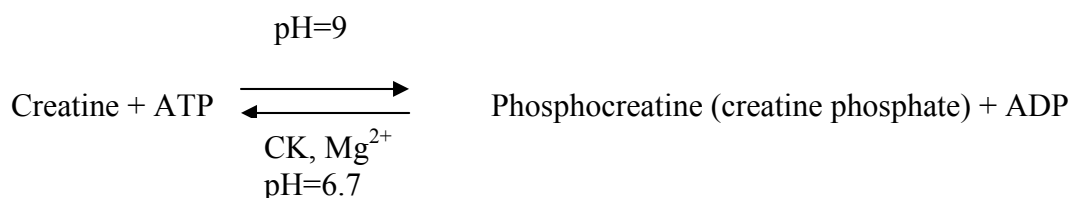
Heart diseases: In myocardial infarction high activity of AST is seen in serum. ALT activity is within normal range or slightly increased in uncomplicated myocardial infarction. Rise in AST is seen within 6 to 8 hours of the onset of chest pain, highest level at 18 to 24 hours and returns to preinfarction levels by 4th to 5th day. There are other superior markers available for myocardial infarction as AST lacks the tissue specific characteristics, as its activity may also increased in diseases of other tissues like liver and skeletal muscles.

Skeletal muscle diseases: AST and occasionally ALT activity levels are increased in progressive muscular dystrophy and dermatomyositis. Level of AST may go as high as 8 times of the normal. There is no increase in the enzyme activity in the muscle diseases of neurogenic origin. Increased AST activity, 2 to 5 times of normal, is also seen after crushed muscle injuries.

In other conditions like pulmonary emboli, acute pancreatitis, hemolytic disease and gangrene the activity of AST is found to be 2 to 5 times higher than the normal activity.

II. Creatine Kinase (CK)

Creatine kinase catalyzes the reversible phosphorylation of creatine by ATP.



The equilibrium position for the reaction is pH dependent. At neutral pH, phosphocreatine has a much higher phosphorylating potential than does ATP, thus favors the reverse reaction. The reverse reaction proceeds 2-6 times faster than the forward reaction under optimum reaction conditions. During muscle contraction ATP is consumed to form ADP, this ADP is again rephosphorylated to ATP by enzyme creatine kinase (CK) using phosphocreatine as a phosphate donor. Phosphocreatine is the major phosphorylated compound present in muscles, eight times more than that of ATP. Enzyme activity is inhibited by excess ADP, urate, cystine and metal ions like Mn^{2+} , Ca^{2+} , Zn^{2+} & Cu^{2+} . Mg^{2+} is required for the activity of CK but excess of it inhibit the CK activity.

The CK is widely distributed and seems to be primarily concerned with ATP regeneration. The activity of CK is highest in striated muscle, heart muscle and brain. The activity in liver and erythrocytes are negligible. Its activity in the serum is unstable due to the oxidation of the sulfhydryl group at the active site of the enzyme. The enzyme is dimeric, composed of 2 subunits namely B (Brain) and M (muscle). Names indicate major tissue of origin. B and M are the product of two different genes. These subunits form three different dimers and form

three different isoenzymes namely BB (CK-1), MB (CK-2) and MM (CK-3). Isoenzymes BB predominates in brain, prostate, gut, lungs, bladder, uterus, placenta and thyroid, MM predominates in skeletal muscle and heart muscle and MB isoenzymes is present in varying degree in heart muscle (25%-46% of CK activity) and some in skeletal muscle. Fourth isoenzymes CK-Mt (mitochondrial), found in the mitochondria between the inner and outer membranes, is different from other forms both immunologically and in electrophoretic mobility.

The CK-3 isoenzyme is normally responsible for almost all CK enzyme activity in healthy people. Serum CK activity appears to be a function of the muscle mass of the individual. Females have lower serum activity than male and that more muscular or well built persons often have higher CK activity than those with slightly built person of the same sex. Normal serum value is 10-50 IU/ L.

Clinical significance

Serum CK activity is elevated in tissue damages involving skeletal muscle, heart muscle, brain injury etc. Elevation of particular CK isoenzymes activity in serum is of diagnostic value.

Heart diseases: CK activity in serum invariably increases after myocardial infarction (MI). CK-2 levels rise 3 to 6 hours after a heart attack. If there is no further damage to the heart muscle, the level peaks at 12 to 24 hours and returns to preinfarction level in 12 to 48 hours. The use of total CK and CK-2 in the diagnosis of myocardial infarction is the most important single application of CK measurements in clinical chemistry. Percentage of CK-2 over total CK activity is valuable in diagnosis of MI. Preinfarction values of CK-2 are usually less than 6% of the total CK activity, but following an infarction values can increase up to 30% depending on the extent of myocardial damage, location of the infarct or the methods used for analysis.

The diagnostic sensitivity of total CK in myocardial infarction is 93-98% and for CK-2 it is nearly 100%. And the diagnostic specificity is 75-85% for total CK and nearly 100% for CK-2 if the level of CK-2 is >6% of total CK.

Elevation of total CK and CK-2 is seen in cardiac trauma following heart surgery which may mask elevation due to intraoperative myocardial infarction. Other cardiac conditions like angina pectoris, cardiogenic shock, electrical counter shock, myocarditis, congestive heart failure, cardiac intra arterial procedures have reportedly shown elevations of total serum CK or CK-2 or both. CK-2 level of <5% or 6% of total CK usually exclude the myocardial damage.

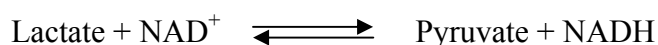
Skeletal muscle diseases: High CK activity is found in all types of muscular dystrophy. In case of Duchenne muscular dystrophy the level may go as high as 50 times that of the normal level. In progressive muscular dystrophy, enzyme activity in serum is highest in childhood and may be elevated long before the disease is clinically apparent, About 50 to 80% of asymptomatic female carriers of Duchenne dystrophy show 3-6 fold elevations of CK activity. Elevation of CK activity is observed in other muscular diseases including malignant hyperthermia. Muscle disease of neurogenic origin may have normal CK activity in the serum.

CNS diseases: In cerebral ischemia , acute cerebro vascular disease, head injury and neurological interventions the level of CK in the serum may increase. In Reye's syndrome, which is characterized by acute brain swelling with fatty infiltration, the CK activity may rise to upto 70 fold of normal level.

Thyroid diseases: There is an inverse relationship with thyroid activity. Elevation of CK activity upto 5 times the normal level is seen in about 60% of the hypothyroid subjects. The major enzyme present is CK-3, CK-2 may go up to 13% of the total CK activity if myocardial involment is there in the hypothyroid subjects.

III. Lactate dehydrogenase (LDH)

Lactate dehydrogenase catalyzes the oxidation of L-lactate to pyruvate. In the reaction hydrogen is transferred from lactate with the mediation of NAD^+ as hydrogen acceptor.



The reaction is reversible and equilibrium strongly favors the reverse reaction i.e. reduction of pyruvate to lactate. LDH is widely distributed in most of the tissues as it is one of the glycolytic enzymes active under hypoxic condition. LDH is composed of four subunits of two types i.e. H and M (H for heart and M for muscles). There are five isoenzymes with different subunit composition named as LDH1 to LDH5.

Isoenzyme	Subunits
LDH1	HHHH
LDH2	HHHM
LDH3	HHMM
LDH4	HMMM
LDH5	MMMM

Isoenzymes are predominantly distributed in the tissue specific manner. LDH1 & 2 are predominantly present in cardiac muscles, kidney and erythrocytes. So care must be taken while taking samples for the LDH analysis for myocardial infarction as sample hemolysis may give erroneous results. LDH4 & 5 isoenzymes are predominant in liver and skeletal muscle. LDH2, 3, and 4 are found in many other tissues like spleen, lungs, endocrine glands, platelets etc.

As compare to the serum, the enzyme activity in the tissues is very high especially in liver, heart, kidney, skeletal muscles etc. Any damage to the tissues may lead to the leakage of the enzyme into the blood and activity rises significantly in the serum. Normal serum value : 60-200 IU/L.

Clinical significance

Heart diseases: In myocardial infarction the level of total LDH activity in serum is 3-4 times that of normal but it may go up to 10 times the normal value. In myocarditis and cardiac

failure with hepatic congestion the serum enzyme activity may be elevated. Moderate elevation of the enzyme activity may be seen in severe shock and anoxia. But no change in serum enzyme activity in angina and in pericarditis. Hemolysis due to any cause may elevate level of LDH1 & 2 in the serum.

Liver diseases: In toxic hepatitis with jaundice serum activity of LDH may be elevated upto 10 times the normal value. Increased activity in serum is also seen in viral hepatitis. Serum LDH5 activity is often elevated in patients with primary liver disease and liver anoxia secondary to decreased O₂ perfusion.

Muscle diseases: The patients with progressive muscular dystrophy often show elevated LDH activity especially LDH5 isoenzyme in the serum. In the later stages of the disease when the muscle mass is lost the LDH level may come down to normal level.

Kidney diseases: In chronic glomerulonephritis, systemic lupus erythematosus, diabetic nephrosclerosis and bladder & kidney malignancies the LDH activity in the urine is found to be elevated 3 to 6 times the normal.

Tumors: Patients with malignant disease show increased LDH activity in serum especially LDH4 & 5. In germ cell tumors like teratomas, seminoma of the testis high level of LDH1 is seen.

IV. Alkaline phosphatase

Alkaline phosphatases are present in almost all tissues of the body. They are membrane bound and are zinc containing metalloenzymes. Alkaline phosphatases are a family of isoenzymes. They hydrolyze a variety of organic phosphate esters transferring phosphate groups from a donor substrate to an acceptor containing a hydroxyl group. The active center of the enzyme contains a serine residue. High levels of enzyme are present in intestinal epithelium (I), Kidney tubules (K), osteoblasts in the bone (B), bile canalicular and sinusoidal membrane of the liver (L), placenta and the lactating breast (P).

Alkaline phosphatases from different sources exhibit three types of activity

1. Hydrolytic R-P + H-OH R-OH + H-P
2. Phosphotransferase R-P + R'-OH R-OH + R'-P
3. Pyrophosphatase R-P-P-R' + H-OH R-P + R'-P

Alkaline phosphatase act on large variety of physiologic and non-physiologic substances. Though the precise natural substrate of alkaline phosphatase in the body is not known, it is associated with calcification and mineralization process in bone and probably in lipid transport in the intestine.

Alkaline phosphatases are a group of true isoenzymes, encoded by at least four different genes: tissue non-specific, intestinal, placental and germ-line ALP. The isoforms derived from the tissue non-specific isoenzyme by post translational modification include the variants of the enzyme found in the liver, bone, kidney and the placenta. Some malignant tumors can produce a placental form of the enzyme called the Regan's isoenzymes.

In serum of normal adults most of the enzyme activity is contributed by liver and nearly half by bone. The respective contributions of these two forms to the total activity are markedly age dependent. Intestine contributes very little amount. The contribution from the kidney is negligible. Placenta contributes a considerable amount during pregnancy. In urine ALP is from renal tissue. The kidneys do not clear the serum ALP. Normal serum value - 3-13 KA units/100ml.

Clinical significance

Physiological bone growth elevates ALP in serum and hence in the sera of growing children enzyme activity is 1.5-2.5 times that in normal adult serum. The level of ALP in the serum of women in the third trimester of pregnancy is 2-3 times more than that of normal level.

Liver diseases: Biliary obstruction due to any cause may elevate ALP level by increasing its synthesis from the hepatocytes adjacent to the biliary canaliculi. This newly synthesized ALP enters the circulation and elevates the enzyme level in the serum. Elevation of ALP in the serum is more with extrahepatic obstruction by stones or by carcinoma head of pancreas than in intrahepatic obstruction. The enzyme level may return to normal on removal of the obstruction. Liver diseases affecting parenchymal cells like infectious hepatitis show only moderate elevation or normal serum ALP levels.

Bone diseases: Bone diseases with increased osteoblastic activity shows increased ALP level in the serum. High ALP levels sometimes up to 25 times the normal value are seen in osteitis deformans (Paget's disease). In Paget's disease there is resorption of bones due to uncontrolled osteoclastic activity and body tries to rebuild bone by increasing osteoblastic activity leading to high ALP level. Moderate increase in ALP level in the serum is seen in osteomalacia, rickets (comes down to normal on treatment with vitamin D), Fanconi's syndrome, primary and secondary hyperparathyroidism. Secondaries in bone from prostate cancer show high serum ALP. Very high ALP levels are present in patients with osteogenic bone cancer.

V. Acid phosphatase

Acid phosphatase include all the phosphatases catalyzing the following reaction at an optimal pH below 7:



Acid phosphatase is an enzyme found throughout the body, but primarily in the prostate gland. The male prostate gland has 100 times more acid phosphatase than any other body tissue. Tissues other than prostate have small amounts of acid phosphatase, including bone, liver, spleen, kidney, and red blood cells and platelets. Acid phosphatases are present in lysosomes, some extra lysosomal acid phosphatases are also found in many cells. Damage to these tissues causes a moderate increase in acid phosphatase levels. Different forms of acid phosphatase are found in different organs, and their serum levels are used as a diagnostic for disease in the corresponding organs. Acid phosphatase from prostate contributes to 1/3rd to 1/2 of the enzyme activity present in the serum of a healthy male. The source of the remainder of

the acid phosphatase in the serum from healthy males and females is not clear. Normal serum value - 1-5 KA units/ 100ml.

Clinical significance

The highest levels of acid phosphatase are found in metastasized prostate cancer. It is of clinical importance to differentiate prostatic and nonprostatic form of acid phosphatase. The prostatic enzyme is strongly inhibited by dextrorotatory tartrate ions, whereas the erythrocyte isoenzyme is not. Formaldehyde and cupric ions inhibit erythrocyte acid phosphatase but not the prostate acid phosphatase. Diseases of the bone, such as Paget's disease or hyperparathyroidism; diseases of blood cells, such as sickle cell disease or multiple myeloma; or lysosomal disorders, such as Gaucher's disease, also show moderately increased levels of acid phosphatase.

VI. γ -Glutamyltransferase (GGT)

GGT comes under the peptidase group of enzymes which specifically catalyzes the transfer of γ -glutamyl group from peptides and other compounds that contain it to the substrate itself, some amino acid or peptide, or even water in which case a simple hydrolysis takes place.

The enzyme acts only on peptides or peptide-like compounds containing a terminal glutamate residue joined to the remainder of the compound through the terminal (5 or γ) carboxyl.

GGT is present in serum which originates primarily from the hepato-biliary system. It is present in all the cells except those in muscle. It is predominantly located in the cell membrane and may act to transport amino acids and peptides into the cell in the form of γ -glutamyl peptides. It may also be involved in some aspects of glutathione metabolism. Normal serum value – 80-180 somogy units /100 ml

Clinical significances

Liver diseases: The activity of GGT in serum is elevated in all forms of liver disease. Highest activity is seen in cases of intra- and post-hepatic biliary obstruction. GGT is more sensitive than ALP in detecting obstructive jaundice, cholangitis and cholecystitis. In infectious hepatitis there is only moderate elevation of GGT activity in the serum. In cases of primary and secondary liver cancer the level of GGT increase earlier and more pronounced than other enzymes of the liver. Increased levels of GGT are seen in heavy drinkers and patients with alcoholic liver cirrhosis. High levels are also found in patients receiving anticonvulsant drugs. Increased levels of GGT are found in almost all the diseases involving liver but are of little value in discriminating different kinds of liver disease. Normal levels of GGT are seen in patients with muscle diseases, children older than 1 year or in healthy pregnant women – conditions in which ALP is elevated. Thus measurement of GGT levels in serum can be used to ascertain whether observed elevations of ALP are due to skeletal disease or reflect the presence of hepatobiliary disease.

Pancreatic diseases: GGT activity may increase in acute as well as chronic pancreatitis and in some cases of pancreatic malignancies especially if associated with hepatobiliary obstruction.

Tumors: Prostate malignancy may at time show increased level of GGT in serum. The irradiation of tumors in cancer patients may be accompanied by a rise in GGT activity. In general if there is increased GGT level in serum of cancer patients there is a chance that the tumor might have metastasize to the liver.

VII. Amylase

Amylases are a group of hydrolases that hydrolyze complex carbohydrates containing α -D-glucose units linked through carbon 1 and 4 located on adjacent glucose residues. Both straight chain and branched polyglucans are hydrolyzed at α -1,4-linkages. The branch points (α -1,6-linkages) are not attacked by the enzyme. There are two types of amylases – α -amylase and β -amylase.

β -amylase are found in plants and bacteria, they are also called exoamylases as they hydrolyze α -1,4-linkages only at the terminal reducing end of a polyglycan chain.

α -amylase are found in humans, they are also called endoamylases as they can hydrolyze α -1,4-linkages anywhere along the polyglycan chain randomly, so the large polysaccharides are hydrolyzed rapidly into smaller molecules like dextrans, maltose, glucose etc.

Amylase require calcium ions and are activated by chloride and bromide, pH optimum is 7.0. The molecular weight of serum amylase range from 55 to 60 kDa.

Amylase is present in many organs and tissues. The highest concentration is found in pancreas. It is synthesized by the aciner cells of the pancreas and then through pancreatic duct it is secreted into the intestinal tract. Salivary gland also secretes amylase in the mouth where the hydrolysis of starch takes place while the food is still in the mouth. The salivary enzymes become inactive when it reaches stomach due to the presence of acid. When food reaches duodenum, pancreatic and intestinal amylase act on the polyglucans present in the bolus and hydrolyze them to produce maltose. Maltose is then acted upon by intestinal maltase and hydrolyzed to glucose. In the lower part of the intestine most of the amylase is destroyed by trypsin, but some amylase activity is present in the feces.

Other than pancreatic and salivary amylase, Amylase activity is present in striated muscle, adipose tissue, lung, ovaries, fallopian tubes, semen and testes. The enzyme is also secreted in milk, colostrums and tears. Tumors of lung and ovary, ascetic fluid due to pancreatic tumor may contain amylase. The serum amylase is mainly contributed by pancreatice (P-type) and salivary gland (S-type) and amylase in urine is derived from the plasma. Even after pancreatectomy the level of serum amylase activity is not reduced much as the salivary glands and other sources contribute to the serum amylase activity.

Macroamylasemia: In the serum of macroamylasemia patients, macroamylase is present. They are probably a complex of ordinary amylase and other high molecular weight plasma proteins like IgA, IgG and other molecules. They are not filtered in the kidneys and increases serum amylase activity by 6 to 8 folds. Amylase activity in the urine is found to be lower than normal. No clinical symptoms are however associated with this disorder. The differentiation of macroamylases from the increased serum amylase in e.g. acute pancreatitis is most simply made by determination of the urinary amylase which will be increased if the amylase is of the usual molecular weight.

Clinical significance

Pancreas: Amylase activity assay in serum and urine are mainly done in the investigation of the pancreatic function and the diagnosis of diseases of the pancreas. In acute pancreatitis, serum amylase activity increased within 2 to 12 hours of the onset of the disease with maximal levels in 12-72 hours and the level returns to normal by the third or fourth day. There is 4 to 6 fold increased in amylase activity above the reference limit. However, up to 20% of the cases may have normal amylase activity in the serum. A significant amount of serum amylase is excreted in the urine, so the rise in serum amylase is reflected in the rise of urine amylase activity.

In quiescent chronic pancreatitis both the serum and urine amylase activity is found to be subnormal.

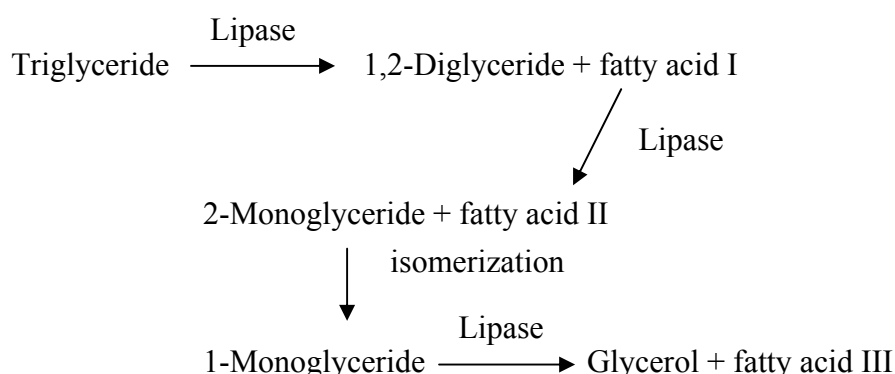
Amylase activity assay is also used in detecting the development of complications following acute pancreatitis like, pseudocyst, ascites, pleural effusion etc. Pancreatic abscess and traumatic lesion to the pancreas, e.g surgical trauma, may cause transient rise in serum amylase activity. Pancreatic cancer leading to the obstruction of the duct may elevate the serum amylase activity

Salivary gland: Any lesion to the salivary gland due to infection (mumps), surgical trauma or any other type of trauma, calculus and tumors may lead to high amylase activity in the serum which is mainly of S-type.

Other causes of hyperamylasemia include renal insufficiency, tumor of the lung and ovary, cholecystitis, ruptured ectopic pregnancy, cerebral trauma, diabetic ketoacidosis.

VIII. Lipase

Lipases are enzymes that hydrolyze glycerol esters of long chain fatty acids. Ester bonds at carbon 1 and 3 only are attacked by the enzyme and from each substrate it produce two moles of fatty acids and one mole of 2-acylglycerol. The enzyme cannot act on 2-acylglycerol due to the steric hindrance but 2-acylglycerol spontaneously isomerizes to 3-acylglycerol which is then attacked by the enzyme and split off the third fatty acid molecule from the glycerol.



Lipase act on the substrate only when it is present in emulsified form. The sequence of lipase acting on the substrate is as follows: first colipase binds to a micelle of bile salt forming colipase-bile salt complex that attaches to the surface of the substrate. To colipase-bile salt

complex the enzyme lipase binds with high affinity. The enzyme lipase is activated by colipase and the lipase action starts efficiently. Normal serum value : <150 units /L.

The main source of lipase is pancreas and the serum lipase is mainly contributed by pancreas, but some lipase is also produced by gastric, pulmonary and intestinal mucosa and also by the tongue.

Clinical significance

Pancreas: Lipase activity in the serum and other body fluid is measured exclusively for pancreatic disorders. In acute pancreatitis, increased lipase activity in the serum is seen after 4 to 8 hours of an attack, peaks at about 24 hours, and come to the normal level by 8 to 14 days. Increased lipase activity parallels that of amylase, but lipase activity may increase sooner and remain longer than that of amylase activity in the serum and the extent of rise is higher with lipase activity. As 20% cases of acute pancreatitis show normal amylase activity, it is necessary to estimate both the amylase and lipase activity in the serum of a patient suspected of acute pancreatitis. Many patient with severe acute pancreatitis develop a pseudocyst in which there is delayed improvement in the clinical condition of the patient. Estimation of lipase show persistent increase in the activity. Serum lipase assay is a more specific diagnostic test in case of patient presenting with acute abdomen to differentiate pancreatic disorder with other acute abdominal disorders like ulcers with perforation, intestinal obstruction etc.

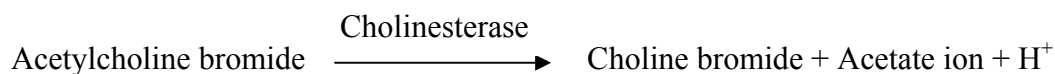
Other conditions in which high serum lipase activity is seen are obstruction of the pancreatic duct by calculus or by carcinoma of the pancreas, acute and chronic renal diseases.

IX. Cholinesterases

Cholinesterases are defined as enzymes that promptly hydrolyze acetylcholine released at the nerve endings. There are two types of enzymes i) acetylcholinesterase and ii) acylcholine acylhydrolase (SChE)

- i) Acetylcholinesterase: It is also known as true cholinesterase or cholinesterase I. It is responsible for the prompt hydrolysis of acetylcholine released at the nerve endings. Acetylcholine degradation is required for depolarization of the nerve so that it can be repolarized in the next conduction event. Acylcholinesterase is found in nerve endings, the gray matter of the brain, spleen, lungs and erythrocytes.
- ii) Acylcholine acylhydrolase: It is also known as pseudocholinesterase or cholinesterase II or benzoyl cholinesterase. Its biological role is unknown but is found in the white matter of the brain, liver, heart, pancreas and serum. The serum enzyme assay of the pseudocholinesterase is clinically useful.

Both the enzymes catalyze the same type of reaction but the specificity towards some of the substrate differs.



Both the enzymes are inhibited by the prostigmine and physostigmine in a competitive manner and are irreversibly inhibited by some organic phosphorous compounds such as

diisopropylfluorophosphate. Other inhibitors are morphine, quinine, phenothiazines, pyrophosphate, bile salts etc.

Genetic variants of the enzyme are found in a small number of populations. There are four allelic forms for the gene (E_1) of the pseudocholinesterase (SChE) enzyme. These are designated as E_1^u , E_1^a , E_1^f and E_1^s . Another genetic locus E_2 is also recognized. The most common phenotype is $E_1^u E_1^u$ or UU. Person with $E_1^a E_1^a$ or AA phenotype are weakly active toward most substrates of cholinesterase and increased resistance to inhibitor dibucaine. $E_1^f E_1^f$ or FF variant is found to be resistant to inhibitor fluoride. $E_1^s E_1^s$ or SS (s for silent) variant is associated with absence of enzyme or with minimal or no catalytic activity if the enzyme protein is present. The AA or FF forms are found only in about 0.3% to 0.5% of the white population and rare in black. Normal serum value: 2.17 – 5.17 IU/ml

Clinical Significance

Cholinesterase activity in serum is useful as an indicator of possible insecticide poisoning. Organic phosphorus compound like parathion, sarin and tetraethyl pyrophosphate which are used as insecticides are inhibitor of cholinesterase activity. Acute organophosphate (OP) poisoning is a significant cause of morbidity and mortality in developing countries including India. Workers in agriculture and in organic chemical industries are exposed to these chemicals and are subject to poisoning by inhalation or by contact.

In India they are freely available in shops and are widely used as insecticides in agriculture and in homes. The anticholinesterase organophosphate compounds - OPCs cause toxicity after their absorption from skin, mucous membranes and respiratory tract following accidental exposure, or from gastrointestinal tract following suicidal ingestion. They are metabolically subjected to hydrolysis by esterases. Although they bind to and interact with a number of enzymes in the human body, yet, it is their action on the enzyme acetylcholinesterase (AChE) that is of clinical importance. These compounds bind to the esteratic site on the acetylcholinesterase molecule phosphorylating the enzyme, leading to inhibition of its normal action. The net result is the accumulation of excess acetylcholine (ACh) at the cholinergic nerve endings all over the body resulting in the characteristic clinical manifestations. Following classical OP poisoning, three well defined clinical phases are seen: initial acute cholinergic crisis, the intermediate syndrome and delayed polyneuropathy (OPIDN). In addition, OPs on chronic exposure affect several of the physiological systems which include central nervous system, neuromuscular junctions, cardiovascular system, metabolic and endocrine systems including reproduction. These effects have been reported both in humans and animals.

Enzyme has importance to the anaesthetist primarily for its rôle in the metabolism of suxamethonium, although other anaesthetic related drugs that this enzyme metabolises are also increasingly important. Clinical applications are primarily centered on subnormal levels of enzyme activity. The decreased activity levels can be caused by inhibitors, reduced biosynthesis, or dysfunctional genetic variants. Changes in enzyme activity should be related to baseline levels because there is wide individual variation as well as methodological variation. Once baseline levels have been established, cholinesterase activity becomes a sensitive indicator of pesticide intoxication and hepatic biosynthetic capacity. A more sophisticated assay, performed in the presence of an inhibitor, is required to detect the atypical genetic variants of serum or plasma cholinesterase.

X. 5' nucleotidase (5'NT)

5' nucleotidase is a phosphatase which is involved in release of inorganic phosphate only from the nucleoside 5' –phosphate, e.g. it acts on adenosine 5'-monophosphate (AMP) to form adenosine and release inorganic phosphate. It is ubiquitously present in all the tissues and is localized in the plasma membrane of the cells in which it is present.

Clinical Significance

Serum 5'NT activity is generally elevated in hepatobiliary diseases, especially with intrahepatic obstruction, but, unlike serum alkaline phosphatase, serum 5'NT activity is not increased in infancy, childhood, pregnancy, or osteoblastic disorders. It can help confirm the hepatic origin of an elevated ALP. Genetic deficiency of erythrocyte pyrimidine 5'NT activity is a common cause of hereditary non-spherocytic hemolytic anemia. Acquired deficiency of erythrocyte pyrimidine 5'NT activity occurs in patients with beta-thalassemia and lead poisoning. 5'NT activity is low in circulating monocytes, increases markedly upon their differentiation to tissue macrophages, and subsequently diminishes during macrophage activation. Lymphocyte ecto-5'NT activity, a plasma membrane marker of cell maturation, is generally low in immunodeficiency states, and undergoes characteristic changes in patients with certain lymphomas and leukemias. In cancer patients, elevated serum 5'NT activity does not always indicate hepatobiliary involvement; in some cases, 5'NT may be released into serum from the primary tumor or local metastases.

Functional tests of Renal, Liver and Gastric fluid

Gastric function tests

The various motor and secretory activities of the stomach can be evaluated by a number of laboratory tests. These include:

- i. Analysis of gastric residue e.g. for blood or lactate
- ii. Analysis of gastric secretions – before and after stimulation
- iii. Determination of intrinsic factor, test for pepsinogen and
- iv. Tests for helicobacter pylori

Some of these tests are more helpful in evaluating specific functions of the stomach for research purposes whereas others provide clinically important diagnostic data. Analysis of gastric acid secretion is central to an understanding of peptic ulcer pathogenesis as the development of ulcers depends on the balance between aggressive factors like gastric acid and factors responsible for mucosal defense. Bacterium *H. pylori* has been shown to be associated with 80% of gastric ulcers and 95% of duodenal ulcers.

Measurement of gastric acidity can be used:

- To detect the gross hypersecretion of acid in some cases of peptic ulcer and in Zollinger Ellison syndrome (caused by gastrin producing tumor)
- In the diagnosis of pernicious anemia (patient show achlohydria)
- In gastric carcinoma 50% patients show achlorhydria.

After an overnight fast a nasogastric tube is introduced in to the patients stomach. The contents (gastric residue) is aspirated and collected. Gastric residue analysis is done by

examining the volume, color, odour, total acidity and occult blood etc. in most normal cases after an overnight fast only a small quantity (20-50ml) of gastric contents is obtained. Volume above 100-120 ml may be considered abnormal either due to oversecretion as in duodenal ulcer and Zollinger Ellison syndrome or due to delayed emptying due to pyloric stenosis. The fresh gastric juice is colorless or light yellow. Bright red. Dark red or brown color can be due to the presence of blood, which may be due carcinoma of the stomach, peptic ulcer or gastritis. Fresh blood may be due to accidental trauma from the nasogastric tube. Disagreeable odour may be produced due to decayed food in the gastric retention. Food particles in the gastric residue indicate either a failure to fast or obstruction to gastric emptying. Organic acid if present are due to bacterial fermentation. Presence of trypsin can be due to regurgitation. Normal pH of the gastric juice is 1.6-1.8.

After the stomach has been emptied, two specimens from the stomach is collected at an interval of 15 minutes without any stimulation, not exposed to any visual, auditory or olfactory stimulation. This basal secretion is analysed for free HCl and the result is reported in mm = millimoles of free acid per hour (Basal Acid Output-BAO). Normal BAO is 1-2.5 mmol/hr. The parietal cell stimulant (pentagastrin, histamine and histalog) is then administered intramuscularly to the patient, then four consecutive 15 minutes samples are collected and time labeled. These are analysed for free HCl and the maximal acid output (MAO) and expressed in mmol/hr. The normal MAO is 20-40 mmol/hr.

Renal function tests

Renal function tests are specialized tests and are advised when medical history, examination and routine tests like urine analysis are suggestive of some renal disease. Estimation of renal function is important in a number of clinical situations, including assessing renal damage and monitoring the progression of renal disease. Renal function should also be calculated if a potentially toxic drug is mainly cleared by renal excretion. The dose of the drug may need to be adjusted if renal function is abnormal.

Renal function tests are only for the analysis of the functional capacity of kidneys. Renal function tests do not give any information about the structural integrity or the structural pathology. For structural details renal imaging and biopsy has to be done.

The functional unit of the Kidney is the nephron, which is composed of the Glomerulus, Proximal convoluted tubules (PCT), loop of henle, Distal convoluted tubules (DCT) and the collecting tubules.

Quantitation of overall function of the kidneys is based on the assumption that all functioning nephrons are performing normally and that a decline in renal function is due to complete functional loss of nephrons rather than to compromised function of nephrons.

Renal function tests may be grouped into (a) those, which assess the glomerular function, and (b) those, which study the tubular function.

Glomerular function tests include:

- Blood Urea
- Blood creatinine
- Inulin clearance
- Creatinine clearance

- Urea clearance
- PAH clearance
- Proteins in urine

Tubular function tests include:

- Urine concentration test
- Urine dilutional test
- Urine acid excretion
- Amino acids in urine

Renal tubules make up 95% of the renal mass, do the bulk of the metabolic work and modify the ultrafiltrate into urine. They control a number of kidney functions including acid-base balance, sodium excretion, urine concentration or dilution, water balance, potassium excretion and small molecule metabolism (such as insulin clearance). Measurement of tubular function is impractical for daily clinical use and performed only when there are specific indications.

Glomerular Filtration Rate: Glomerular filtration rate (GFR) is the rate (volume per unit of time) at which ultrafiltrate is formed by the glomerulus. Expressed in ml/min Normal GFR ~125ml/min

Renal Plasma Flow: Volume of plasma flowing through the kidney per min. Expressed in ml/min. 25% of the total cardiac output.

Filtration fraction: Fraction of renal plasma flow which is filtered through glomeruli. Expressed as percentage.

Clearance: Clearance of a substance is the volume of plasma cleared of the substance per unit time. It is expressed in ml/min.

$$\text{Clearance (C)} = \frac{U \text{ (mg/dL)} \times V \text{ (ml/min)}}{P \text{ (mg/dL)}}$$

where U is the urinary concentration of a marker x , V is the urine flow rate and P is the average plasma concentration of x .

Substance filtered neither reabsorbed nor secreted	Clearance = GFR	Inulin
Substance filtered, reabsorbed and secreted	Clearance $\approx$ GFR	Uric acid
Substance filtered and partially reabsorbed	Clearance < GFR	Urea
Substance filtered, secreted and not reabsorbed	Clearance > GFR	PAH

BUN (Blood Urea Nitrogen): Plasma Creatinine (P_{Cr}) ratio
Normal BUN/ Plasma Creatinine ratio $\approx$ 12-16: 1

High BUN/P_{cr} ratio

prerenal azotemia
 high protein diet
 catabolic states (e.g. sepsis)
 gastrointestinal bleeding
 medications (e.g. corticosteroids)

Low BUN/P_{cr} ratio

severe liver failure
 low protein diet
 anabolic states
 rhabdomyolysis

Normal Values:

- Plasma creatinine – 0.9-1.3 mg/dl (men), 0.6-1.1mg/dl (women)
- Plasma Urea – 15-40 mg/dl

Liver function tests (LFT)

The liver plays a key role in a variety of metabolic, synthetic and excretory functions. Abnormalities in liver function tests reflect not only damage to the hepatic cell, but also give clues to the site, extent and nature of the pathological process. The routine LFTs include:

- 1) Serum bilirubin (Total and Conjugated)
 Urinary bilirubin, urinary urobilinogen
- 2) Serum enzymes:
 - a) Alanine Aminotransferase (ALT)/ SGPT
 - b) Aspartate Aminotransferase (AST)/SGOT
 - c) Alkaline phosphatase
 - d) Gamma glutamyl transferase (transpeptidase)
 - e) 5' nucleotidase
- 3) Serum albumin and total proteins
- 4) Prothrombin time, clotting time

Specialized tests include those for viral markers(eg HbsAg), immunological markers and inherited metabolic disorders of the liver (eg Wilson's disease).

Jaundice is the commonest mode of presentation in patients with liver diseases. Jaundice is a clinicopathological condition characterized by yellowish discolouration of the skin and mucous membrane, sclera, palate and nail beds caused by deposition of bilirubin in the tissues, when the level of which is usually more than 2 to 2.5 mg/dl in the serum. The normal level is 0.3-1 mg/dl. Hyperbilirubinemia occurs when levels are over 1mg/dL (17.1micromoles/L).

Bilirubin is formed by the reticulo-endothelial cells of the liver from ferroporphyrin ring obtained mainly from Hb of erythrocytes and also from myoglobin and iron containing enzymes. Bilirubin exists in blood in either conjugated or unconjugated forms. Normally, most of the bilirubin found in blood is of the unconjugated form. Conjugation of bilirubin occurs in the liver and the conjugated form, which is water soluble, can pass through in the urine whereas the unconjugated form cannot.

Bilirubin is excreted in the bile mainly in the form of bilirubin diglucuronide. In the intestine, it is converted to urobilinogen by the action of bacterial flora. Some of the urobilinogen gets reabsorbed and undergoes enterohepatic circulation as well as excretion by the kidneys. The rest is excreted in the faeces as stercobilinogen. Urobilinogen gets oxidised to urobilin by

atmospheric oxygen after excretion in the urine and so also is stercobilinogen oxidised to stercobilin.

Jaundice again can be divided into three types -

a) **Haemolytic**: Due to excessive haemolysis; usually results in a predominant increase in unconjugated bilirubin; also termed retention hyperbilirubinemia or acholuric jaundice e.g. sickle cell anemia, thalassemia, drug induced jaundice, resorption of a hematoma.

b) **Hepatocellular**: due to damage to hepatocytes; results in increases in both the direct and indirect fractions; depending on the nature and extent of the disorder, the predominant increase may be in either the unconjugated form (e.g. neonatal jaundice- levels over 20mg/dL cause kernicterus, Gilbert's disease, the Crigler-Najjar syndromes) or the conjugated form (e.g. viral hepatitis, drugs, alcohol. Sepsis, congestive cardiac failure, Dubin Johnson and Rotor syndromes)

c) **Obstructive**: Due to obstruction of flow of bile (eg stone, stricture or tumour in the bile tract). Predominant increase in conjugated bilirubin.

This classification of jaundice is not strictly mutually exclusive as any given patient may have features of more than one type of jaundice depending on the nature and extent of disease(s).

Liver function tests in different types of Jaundice

	Haemolytic	Hepatocellular	Obstructive
a) Pigment metabolism			
Plasma bilirubin	Indirect	Usually biphasic	Direct
Urine bilirubin	No	Variable	+
Urine urobilinogen	++	+/-	Nil (if complete)
b) Plasma alkaline Phosphatase(KA units)	3-13	<30	>30

Determination of Serum Bilirubin

Normal values: 0.3-1mg/dL;

Hyperbilirubinemia when > 1mg/dL

Jaundice manifested > 2-2.5 mg/dL

Measurements of some serum enzymes are commonly done in patients with liver disease. Normally these enzymes are involved in metabolism in intact hepatocytes and that is why their levels are extremely low in plasma. When the liver is damaged, the enzymes come out of the hepatocytes into the circulation giving high values, which are diagnostically important. These enzymes are not specific for liver but abnormal values give an idea about the nature and extent of disease.

The following enzymes may be used in the assessment of hepatobiliary functions:

1. Serum Transaminases (ALT, AST)
2. Serum alkaline phosphatase (ALP)
3. Gamma- glutamyl transpeptidase (transferase) (GGT)
4. 5'-nucleotidase (5'-NT)

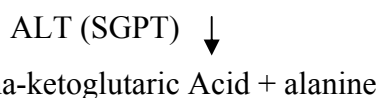
Serum Transaminases

Aspartate aminotransferase (AST) also known as Serum glutamate oxaloacetate transaminase (SGOT) and alanine amino transferase (ALT) also known as serum glutamate pyruvate transaminase (SGPT) catalyse the following reactions.

1. Glutamic acid + Oxaloacetic Acid



2. Glutamic acid + Pyruvic Acid



For both reactions, pyridoxal-5'-phosphate is the cofactor.

Both the enzymes are present in the cytosol of the hepatocytes. AST is also found in the mitochondria. ALT is present primarily in the liver and to a lesser extent in kidney and skeletal muscle. AST is found in all body tissues especially heart, liver and skeletal muscle.

Neither enzyme is specific for the liver but as ALT is found in much higher concentration in liver than in other organs. Increase in its activity indicates that the liver is damaged. Therefore, in absence of acute necrosis or ischaemia of other organs such as myocardium, high serum ALT and AST activity suggest liver cell damage.

Normal value (colorimetric methods) serum ALT = 6-30 U/L
serum AST = 8-34 U/L

In viral hepatitis, there is generally increased transaminase activity (ALT, AST) of 10 to 100 times the normal value in the jaundiced (acute viral hepatitis) phase (upto 4000 U/L), after which the activity falls rapidly. Equally high transaminase activity occurs in acute hepatitis due to drugs (eg steroids), acute circulatory failure and exacerbation of chronic active hepatitis. In alcoholic hepatitis, serum AST tends to be high than ALT.

Serial determination of serum ALT and AST are helpful in following the course of a patient with liver disease with acute or chronic hepatitis. Moderate elevations of ALT is seen in cirrhosis and metastases. Minimal rise may also be seen in biliary tract obstruction.

Liver is richest in ALT. ALT levels do not rise proportionally in myocardial infarction as compared to AST levels. Low values of ALT have no clinical significance.

Elevated levels of Alkaline phosphatase (ALP), a GPI anchored membrane bound enzyme usually indicate biliary tract obstruction, though non-hepatic causes for a rise in ALP have to be excluded. Increased levels of 5'-NT are seen if there is hepatobiliary damage and can help confirm the hepatic origin of an elevated ALP. GGT elevations tend to correlate with rise in ALP. High GGT levels are usually seen in alcoholic liver disease.

Enzymes increased in serum in liver damage:

1. Serum Transaminases (ALT, AST)
2. Lactate Dehydrogenase
3. Isocitrate Dehydrogenase

Enzymes increased in serum in cholestasis:

1. Alkaline phosphatase
 - Location / Tissues (non-hepatic causes)
 - Regurgitation and increased synthesis.
2. 5' Nucleotidase
 - highly specific and sensitive marker for intra and extra-hepatic cholestasis
 - Increased levels parallel increased levels of ALP
 - Highly specific for hepatobiliary disease. Not increased in children or pregnancy (unlike ALP).
3. Gamma Glutamyl Transferase (GGT)
 - Transfers γ -Glutamyl group from one peptide to another.
 - Increased GGT activity in any and all forms of liver disease specially intra and extra hepatic biliary obstruction.
 - GGT is the most sensitive enzymatic indicator available at present for hepatobiliary disease
 - GGT rises very early but cannot discriminate between different kinds of liver disease.
 - Elevated levels of GGT are seen in Alcoholic liver disease.

Tests for synthetic function:

1. Total Serum Proteins (Albumin, A/G ratio).
2. Prothrombin Time.
3. Blood Ammonia.

Suggested Readings

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