Clinical significance of enzymes

The relative abundance of certain enzymes or their isoenzymes in serum has found application in the diagnosis of several diseases as mentioned below.

Alkaline phosphatase

Measurement of the activity of alkaline phosphatase in serum in the investigation of bone diseases constituted one of the earliest applications of enzyme tests in diagnosis and has remained one of the most useful. The value of serum alkaline phosophatase assays in various disorders of bone has been confirmed by more than 50 years' experience, while isoenzyme studies have demonstrated that bone is indeed the source of the raised serum enzyme activity in these conditions. In contrasts, no other enzyme test has emerged of comparable value in bone diseases, inspite of the systematic attention which has been devoted to the development of diagnostic enzymology in recent years.

Raised serum alkaline phosphatase was shown to accompany rickets of various aetiologies, Paget's Disease and hyperparathyroidism with skeletal involvement.

y-**glutamyl transferase is probably the most sensitive enzymic test of liver function yet described.**

The normal range of serum alkaline phosphatase activity in adults is usually taken as **3-13 King-Armstrong units/100 ml.**

The serum alkaline phosphatase activity of the new-born infant is rather above the normal adult level, and it rises rapidly to as much as two and a half

to three times the adult upper limit of normal during the first year of life. The activity falls somewhat by the end of the second year to values which are of the order of one and a half to two and a half times of 20-30 King - Armstrong units/100ml. These elevated values are maintained approximately constant throughout childhood and early adolescence with perhaps some upturn at the onset of puberty, typically declining to adult levels during the later teenage years.

When hepatobiliary disease is suspected, it is advisable to resort to an alternative enzyme test. The activities of several enzymes in serum reflect impaired biliary function in ways similar to (but not completely identical with) the behaviour of alkaline phosphates in this situation. These enzymes typically show normal activities in osteoblastic bone diseases. **Two such enzymes, i.e., 5'- nucleotidase and Y-glutamyltransferase,** have been shown to exhibit serum activities within the adult normal range in children and their use has therefore been recommended as a liver function test in young patients.

The highest serum alkaline phosphatase levels are generally encountered in Paget's disease of bone (Osteitis deformans), and the raised enzyme activity is often the only abnormality in the composition of the serum seen in this condition. The activity in serum may reach levels of 100-200 King-Armstrong units/100ml or more, **the higher values being associated with cases in which a greater proportion of the skeleton is involved.**

While, determination of serum alkaline phosphatase activity is of great value in the detection of Osteomalacia and rickets, the same

is not true of hyperparathyroidism, in which an increased serum alkaline phosphatase is only seen in those cases in which bone changes are present. Successful treatment of hyperparathyroidism (e.g. by the removal of a parathyroid adenoma) is followed by a fall in serum alkaline phosphatase activity which may, however be preceded by an initial rise due to healing of the bony lesions : this contrasts with the immediate fall in serum calcium Infiltration of the bones by malignant disease is accompanied by a rise in serum alkaline phosphatase activity whenever the process stimulates osteoblastic activity.

ISOENZYMES OF ALKALINE PHOSPHATASE (ALP)
On careful electrophoresis of plasma, four isoenzyme bands of ALP can be distinguished (liver, placenta, intestine, bone), but unfortunately there is a major difficulty in distinguishing the bone enzyme from the liver enzyme, as they migrate very close together in the support systems used. With these difficulties ALP isoenzyme detection has so far had to remain as a semi research procedure.

Acid phosphatase

Acid phosphatase is an important enzyme and is found to be in abundance in adult prostate and seminal fluid, while significant amounts are found in several other tissues such as red cells, liver, kidney, bone, spleen and pancreas.

Normal range

1 to 3.5 K.A.U./100ml or

1.8 to 6.5 I.U./litre

Most of the activity in normal serum is nonprostatic in origin and is found in both sexes at all stages. Less than 0.8 K.A. units is tartarate labile and so represents prostatic fraction.

Elevation

Clinical significance of acid phosphatase is chiefly in metastatic cancer of prostate, both in detection and following the progress of disease. **Raised values mostly between 5 to 50 K.A.U. are seen in over 75% cases of metastasizing prostatic carcinoma. Sometimes, values over 100 and very rarely even over 1,000 may be found.** Most of the increased enzyme activity is tartarate labile. Very high levels may be found in some cases of malignant prostate with secondaries (most often in bones).

A small increase has also been found in acute retention and following prostate massage, but values soon return to normal. Similar increases are noticed in Paget's disease and carcinoma of breast but in such cases the determination is not of much clinical significance.

If the increase in tartarate labile fraction is not significant and the total level is found raised, the increase may be due to red cells or other sources of acid phosphatase.

Isoenzymes of acid phosphatase

By polyacrylamide gel electrophoresis, various isoenzymes designated arbitratily as 0, 1, 2, 3, 3b, 4 and 5 have been identified from different sources in normal and disease conditions. Zero represents the slowest moving and 5 the fastest moving fraction. Most of these isoenzymes are labile even at

the room temperature and become inactivated. Therefore, in the serum only stable isoenzyme is found.

The isoenzyme pattern in the leukocytes found is as follows :

Isoenzymes	**Sources**
1, 2, 4	in neutrophils
1, 4	in monocytes
3	in lymphocytes and platelets
3b	in primitive blasts
5	in reticulum cells of leukaemic reticuloendothelosis

ISOENZYMES OF ACID PHOSPHATASE (ACP)
At the most, there do exist 14 isoenzymes on electrophoresis. One fiend clear cut 14 bands on electrophoretogram. **The only isoenzyme of clinical importance is prostatic acid phosphatase.**

Normal plasma contains a tartarate resistant isoenzyme 5.

In variety of diseases, other isoenzymes may appear in plasma.

In metastatic prostatic cancer-changes in plasma enzyme activity are due to the presence of isoenzyme 2, which is tartarate labile.

Many acid phosphatase isoenzyms are cell specific i.e. derived from a specific type of cell.

AMINOTRANSFERASE (Transaminase)

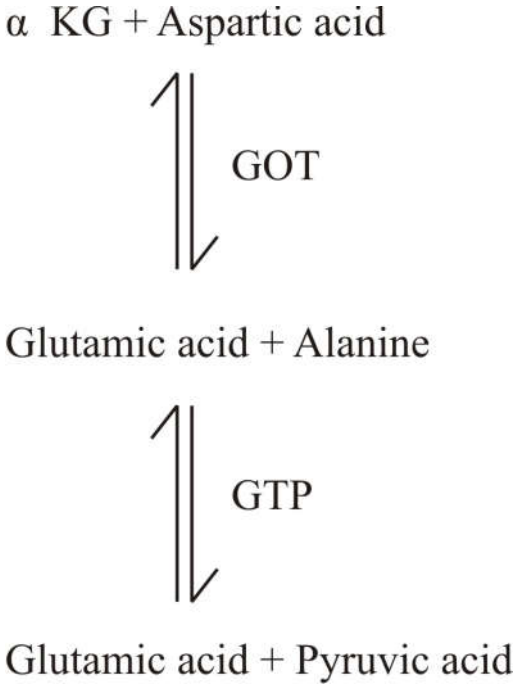
The enzymes which are involved in the transfer of an amino group CH_2-NH_2) from an alpha amino acid to alphaketo, acid are called as transaminases and the process is called as transamination reaction is an important step in the metabolism of amino acids.

Two enzymes occur in human tissues which catalyzes reaction of this type

1) Aspartate amino transferase, popularly known as GOT **(glutamine-oxaloacetic transaminase)** and

2) Alanine amino transferase, Transamination **popularly known as GPT (glutamine Pyruvic transaminase)**

Both transaminases are widely distributed in human tissues, GOT activity almost always being greater than GPT activity. Serum GOT and GPT catalyse the following reactions of transamination respectively



When ECG findings are not very clear. GoT rises rapidly after myocardial Infaraction.

Interpretation

The normal range for serum GOT is 4-17 IU/I or 8-40 units/ml and for GPT 3-15 IU/ OR 5-35 units/ml. According to Wroblemsky in the new born values upto 120 units for GOT and 90 units for GPT must be considered normal. Determination of GOT is particularly useful in myocardial infarction (M.I.) When peak values which may be 2-20 times of normal, are reached within 24-48 hrs and return to normal levels typically with 3-5 days.

Elevated level of GOT has been observed in almost 97% clinically proved cases of M.I.

Normal value have been reported in heart condition without infarction, such as angling and pericarditis, in patients with pulmonary embolism and also in patients with acute abdominal attacks. Foulk and Fleisher found an increase in over 50% patients with acute pancreatitis. SGPT is not usually in M.I. unless the lesion in a large one or there is associated liver damage.

Both the enzyme exhibit raised levels in hepato cellular damage due to hepatotoxic drugs, infective hepatitis and primary and secondary liver cancer.

Raised levels are also seen in **Lannec's cirrohsis,** biliary cirrohsis and obstructive jaundice In infective hepatitis, the increase is particularly marked and highest value are obtained. In early stage GOT is more increased than GPT but SGPT exceeds SGOT in advanceds stage. In infective hepatitis estimation of serum transaminases is the most sensitive diagnosis index.

Very high value are obtained in toxic hepatitis due to carbon tetrachloride poisoning. Serum transaminases particularly SGOT gets elevated in certain muscular disorder eg progressive muscular dystrophy, dermatomyositis and also following extensive muscle trauma, but is usually normal in progressive muscular atrophy gravis and rheumatotoid arthritis.

Recommendations on the use of Serum Enzyme Assays in the diagnosis of Acute MYOCARDIAL INFARCTION

(1) A single set of cardiac enzyme values in the emergency room is not sufficiently sensitive to exclude myocardial infarction. Although a single, **markedly positive CK-MB value will greatly increase the probability of acute infarction,** data are insufficient to support or reject a policy where low rise patients who other wise would be sent home, would be observed until one or more CK-MB value are obtained.

(2) If a myocardial infarction is inspected the sample of total CKMB and CK levels should be measured on admission and about 12 and 24 hrs later, although condensed version of this strategy may ultimately prove to be equally efficacious and more cost effective. If MI may have occured more than 24 hrs before admission, and if Ck and Ck-MB levels are not diagnostic, a total LDH levels should be ordered. If the total LDH level is elevated an assay of LDH isoenzymes should be obtained. If the first LDH-1 / LDH-2 ratio is only slightly less than 1-0 a second assay is propably indicated.

(3) If the chest pain occurs after admission, CK and CK-MB assays should be done at 0, 12 and 24 hours.

(4) Routine use of enzyme assays other than those for CK, CK-MB and **LDH iso enzyme** is not recommended.

(5) If more than 2 hours may pass before CK, iso enzyme, will be pass assayed, the serum sample should be preserved on ice.

OTHER LABORATORY MEASUREMENTS

BLOOD SUGAR -

Hyperglycemia occurs frequently following AMI (acute myocardial infarction) not only in diabetic patients in whom keto acidosis may be precipitated, but also (with a lower frequency) in non diabetics, in whom several weeks may elapse before carbohydrate tolerance returns to normal.

SERUM LIPIDS : These are often determined in patients with AMI. However, the results may be misleading, since numerous factors that canalter the values are operating at the time of the patients admission to the hospital, for example **stress increases serum cholesterol, whereas recumbacy (the state of taking rest by lying down) decreases** it. Serum triglyceride are affected by calorie intake, intravenous, and recumbency.

During the first 24-48 hours after admission, total cholesterol and HDL cholesterol remain at or near baseline value but generally fall precipitatously after that. The fall in HDL cholesterol, after AMI is greater than the fall in total cholesterol thus the ratio of total cholesterol to HDL cholesterol is no longer useful for risk assessment eary after MI Therefore, unless value are obtained very early in patients admitted for AMI it is the best to defer determination of serum lipids levels until at least 8 weeks, after the infarction has occurred.

SERUM AMYLASE : Amylase is a starch splitting enzyme which hydrolyses starch and other high molecular dextrin to maltose at an optimum ph of 71. It is present in the pancreas and parotid gland. The major activity of this enzyme, called amylopsin, is in pancreatic juice, some is also found in saliva.

INTERPRETATION : Normal range of serum amylase is given as 80-180 Somogyi units/ 100 ml. **The clinical significance of this enzyme chiefly lies in the diagnosis of acute pancreatitis in which value frequently exceed 2000 units.** Usually activities over 550 units strongly suggested a diagnosis of acute pancreatitis. The rise is rapid and transient, reaching a peak within the first 12-24 hrs after the onset and returning to normal usually in 2 to 3 days.

In chronic disease of the pancrease whether due to neoplasm or due to chronic pancreatitis, such high values are not seen. (rarely exceeds 200 units 100ml) In these conditions either duct obstruction is more gradual or progressive destruction of secreting tissues largely counteracts its effects.

High activities may also occasionally occur in variety of condition in which pancreatic damage is secondary to other abdominal disturbance such as perforated gastric ulcer, peritonitis, intestinal obstruction. In these conditions values over 500 units may be found. These usually develop 5 to 10 days after the onset of symptoms and appear to be due to increased pressure on pancreatic duct.

ISOENZYMES OF AMYLASE : Although techniques are becoming available for the separate estimation of amylase isoenzymes {pancreatics

salivary} the measurement of isoenzymes is unlikely to resolve the major diagnostic problem of differentiating acute pancreatitis from non pancreatic abdominal emergencies (perforated peptic ulcers gut, obstruction etc). This because in the later the, situation, pancreatitis amylase that is normally present in the gut lumen leak through the intestinal wall into the peritoneal cavity and from there it is absorbed into the blood stream.

Drugs such as morphine which causes contraction of sphincter odd increase serum amylase.

ISOENZYMES OF PNOSPHOKINASE (CK or CPK)

Creatinine kinase (CK) OR creatinini phosphokinase (CPK) catalyses the inter conversion of phosphate creatine (or creatine phosphate) to creatine.

$$\text{Phosphocreatine} \xleftarrow[\text{ADP} + P_1 \quad \text{ATP}]{\text{CPK}} \text{creatine}$$

CPK exists as three iso enzymes. Each iso enzymes is a dimer composed of two subunits - M(muscle) or B (brain) or both

ISOENZYMES	SUBUNITS	TISSUE OF ORIGIN
CPK_1	BB	Brain
CPK_2	MB	Heart
CPK_3	MM	Skeletalmvscle

In healthy individual, the iso enzymes CPK_2 (MB) is almost undetectedable in serum with less than 2% to total CPK. After the myocardial infarction (MI). Within the first 8 hrs CPK_2 increase in the serum to as high as 20% (against 2% normal) pk2 iso enzymes is not elevated in skeletal muscle

disorders. Therefore estimation of CPK_2(MB) is the earliest reliable indication of Myocardial infarction.

ISOENZYMES OF ALKALINE PHOSPHAT

As many as six isoenzymes of alkaline phosphatase have been identified. The enzymes is a monomer, the isoenzymes are due to the difference in the carbohydrate content (sialic acid residues). The most important ALP isoenzymes are α1 - α2 ALP, α2 heat labile, ALP, pre-β-ALP, Y-ALP etc.

ISOENZYMES OF ALCOHOL DEHYDROGENASE

Alcohol dehydrogenase (ADH) has two heterodimer isoenzymes. Among the white Americans and Europeans $\alpha\beta$ isoenzyme is predominant whereas in Japanese and Chinese (oriental) $\alpha\beta_2$ is mostly present. The isomer $\alpha\beta_2$ more rapidly converts alcohol to acetabldehyde.

Accumulation of acetaldehyde is associated with tachycardia (incrrease in heart rate) and facial flushing among Orientals which is not commonly seen in whites. It is believed that Japanese and Chinese have increased sensitivity to alcohol due to the α β_2 isoenzyme of ADH.

ISOCITRATE DEHYDROGENASE :

It has four isoenzymes. ICD-$_1$, ICD-$_2$, ICD-$_3$, and ICD-$_4$. Increased activity of ICD in the serum suggests liver disease, particularly related to toxicity, inflammationor malignancy.