Clinical Enzymology

• Clinical treatment of enzymes:
  – Tissue Enzymes
  – Plasma Enzymes

• Tissue enzymes—Local action
• Plasma enzymes—Cosmopolitan action
Plasma Enzymes

- Heterogeneous distribution
- Clinically significant
- Two classes
  - Functional plasma enzymes
  - Non-Functional plasma enzymes
Functional Plasma Enzymes

- Present in plasma in higher concentrations than in tissues
- Have known functions
- Their substrates are present in blood
- Mostly synthesized in liver
- Usually decreased in case of diseased condition
- Examples: clotting factors, lipoprotein lipase, etc.
Non-Functional Plasma Enzymes

- Present in plasma in lower concentrations than in tissues
- No known functions in plasma
- Their substrates are absent from blood
- Synthesized in liver, heart, skeletal muscles, brain, etc.
- Usually elevated in diseased conditions

Examples: AST, ALT, CPK, LDH, ACP, ALP, etc.
Non-Functional Enzymes

That’s a misnomer for Biochemists!!
Isoenzymes

• Isoenzymes (also known as isozymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction.

• Believed to be originating from closely linked genes or from multiple gene loci.

• Evolution from a single form possibly due to long-term mutations.

• They vary with respect to their kinetic parameters, electrophoretic mobility, and localization.
Unity in Diversity!!

- Importance due to their distribution (localization)
- Independent action
- Examples: LDH, CPK, etc.
So What’s the Catch?

• They are of diagnostic value
• Can be differentiated from each other
• Can be clinically quantified in the lab
• Identify disorders related to specific areas in the body

Enzyme Concentration

• Bedrock of clinical enzymology
• Knowledge of standard values is the key!
• Enzyme concentrations determined at
  • Plasma level
  • Serum level
  • Cellular level
• Depends on various factors
Factors Affecting Enzyme Concentration

- Enzyme formation
- Enzyme release into circulation
- Enzyme clearance
- Cellular leakage of enzymes
Enzyme Pattern in Health & Diseases

• Variation from normal values indicate disease/disorder.

• Clinical impacts of:
  • Plasma lipase
  • Amylase
  • Cholinesterase
  • Phosphatases (acid and alkaline)
  • Aminotransferases (AST, ALT)
  • Lactate dehydrogenase
  • Creatinine phosphokinase
Lactate Dehydrogenase

• Conversion of pyruvate to lactate in a reversible manner
• Isoenzyme, exist in 5 forms.
• Normal values: 60–250 IU/L
• Isoenzymic variations in different disease conditions.
Lactate Dehydrogenase Fact File

- Additional form: LDH$_x$ in male genitalia.
- LDH is commonly addressed based on their location as hepatic LDH, muscle LDH, cardiac LDH, and so on.
- LDH-2 – Most prominent in normal serum.
Lactate Dehydrogenase Fact File

• Made up of different ratios of H and M chains
• LDH-1 has the greatest negative charge.
• LDH-1, 2 are heat resistant (up to 60°C) while LDH-4, 5 are heat labile.
• LDH-5 (Cardiac LDH) is inhibited by urea.
• LDH-1, 2 prefer oxo-butyrate over pyruvate but it is not so in liver LDH
LDH & Diseases

• Important biological markers
• Diseases of live, heart, muscle, and malignancies can be detected.
• Elevated in myocardial infarction within 12 h and peaks around 48 h. Returns to normal in 8–14 days. (Nonspecific)
• Also elevated in leukemias, carcinomas, renal and hepatic cell necrosis, muscular dystrophy, etc...
Creatine Phosphokinase

- Conversion of creatine to phosphocreatine in an energy-dependent reaction
- Exists in 3 isoenzymic forms
- Exist as dimers
- Can be differentiated on an electrophoretic gel, or in an IEC column
- Normal values: 4–60 IU/L
Creatine Phosphokinase Fact File

• Humans – 3 isoenzymic forms
• Exist as dimers (Brain and Muscle forms)
  – CPK-1: BB
  – CPK-2: MM
  – CPK-3: MB
• Differ in electrophoretic mobilities (BB is fast moving and MM is slow moving)
• Can be resolved by electrophoresis &
Atypical forms of CPK

- CK-Macro
  - CK-BB with IgG/IgA
  - CK-MM with lipoproteins
- CK-Mi (Mitochondrial CK)
  - 2 main forms: CK-Mi a and CK-Mi b
  - Exist in dimeric/octameric forms
  - Serves as marker for cellular damage
CPK & Diseases

• CPK-1:
  – Injury to lungs or brain (e.g., brain injury such as trauma, stroke, or bleeding in the brain, lung injury due to a pulmonary embolism, brain cancer, electroconvulsive therapy, pulmonary infarction, seizures

• CPK-2:
  – Levels rise 3–6 h after a heart attack (myocardial infarction).
  – If there is no further damage to the heart muscle, the level peaks at 12–24 h and returns to normal 12–48 h after tissue death.
  – Elevation is observed in myocarditis (inflammation of the heart muscle usually due to a virus), electrical injuries, trauma to the heart, heart defibrillation, open heart surgery, etc.
CPK & Diseases

• CPK-3:
  – Elevation is observed in crush injuries of skeletal muscle, multiple intramuscular injections, muscular dystrophy, myositis (skeletal muscle inflammation), post-electromyography, recent seizures, recent surgery, rhabdomyolysis (skeletal muscle damage due to drugs or prolonged immobilization), and strenuous exercise
Alkaline Phosphatase

- Hydrolases: Catalyze the splitting of phosphoric acid from monophosphate esters
- Exists in several isoenzymic forms
- Six forms identified; 4 of them are key forms: hepatic, bone, placental, and intestinal isoenzymes
- Differentiated by electrophoresis, chemical inhibition, and heat inactivation assays.

Normal level: 23–92 IU/L
Alkaline Phosphatase Fact File

• 4 Key isoenzymic forms:
  – Hepatic ALP
    • Greater electrophoretic mobility
    • 2 forms: $\alpha_1$ (faster form) and $\alpha_2$
    • Predominant in adults
  – Bone ALP
    • Mobility close to hepatic ALP.
    • Predominant in children
Alkaline Phosphatase Fact File

- Placental ALP
  - Follows bone ALP
  - Heat stable. Resists denaturation at 65°C for up to 30 min

- Intestinal ALP
  - Slowest mobility; follows placental ALP
  - More common in people with B and O blood groups
2 atypical forms:

- Regan isoenzyme
  - Mobility is similar to bone isoenzyme
  - Heat stable
  - Inhibited by L-Phe

- Nagao isoenzyme
  - Variant of Regan isoenzyme
  - Inhibited by L-Leu

Oncogenic markers: Carcinoplacental isoenzymes
ALP and Diseases

• Non-specific marker enzyme
• Observed to be elevated under conditions of:
  – Hepatic damage (e.g., liver cirrhosis, hepatocarcinoma, hepatobiliary diseases like obstructive jaundice, etc.)
  – Osteoblastic activity in children
  – Rickets, osteomalacia, Paget’s disease
  – Hyperparathyroidism
  – Last 6 weeks of pregnancy
  – Oncogenic markers
• Observed to be decreased during
  – Defective calcification
  – Anaemia
  – Scurvy
Acid Phosphatase

• Hydrolases: Catalyze the splitting of phosphoric acid from monophosphate esters
• Occurs in 2 forms:
  – Prostatic ACP
  – Non-prostatic ACP
• It has its maximum activity around pH 5.6
• Normal value: 0.6–3.1 K.A/dl
Acid Phosphatase
Fact File

• 2 forms
• Prostatic ACP is found in the prostate and also in other tissues like the spleen, kidneys, liver, and the pancreas
• Non-prostatic ACP is observed in the erythrocytes and the leukocytes
• Extremely heat labile
• Labile in the presence of tartarate
ACP & Diseases

• Important marker enzyme for prostate cancer
• Moderate elevations observed in
  – Paget’s disease
  – Hyperparathyroidism
  – Breast cancer
  – Gaucher’s disease
  – Hemolytic anemia
  – Myelocytic leukemia
Serum Glutamate Oxaloacetate Transaminase

- Transamination: Asp and $\alpha$-KG to Glu
- Also known as aspartate transaminase (AST/SGOT)
- Concentrated at the myocardium
- Serves as a marker for myocardial infarction and other cardiac diseases
- It is also non-selectively elevated in damages associated with the liver, skeletal muscle, kidney, and erythrocytes tissues

Normal values: 4–17 IU/l
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Behavior in Myocardial Infarction

• SGOT levels rise sharply within the first 12 h of infarction
• Peak achieved at 24 h
• Normal values are restored in 3–5 days
• Levels depend on
  – Size of infarct
  – Recurrence of infarction
• Levels exceeding 350 IU/L is fatal
• Level is to be maintained within 50 IU/L
Serum Glutamate Pyruvate Transaminase

• Transamination: Ala and $\alpha$-KG to Glu and Pyr
• Also known as alanine transaminase (ALT/SGPT)
• Concentrated at the hepatocytes
• Serves as a marker for hepatic disorders such as liver cirrhosis, hepatic jaundice, hepatocarcinoma, hepatitis, etc.
• It is also increased in inflammatory diseases, lymphoblastic leukemia, alcoholic liver, etc.

Normal values: 3–15 IU/L
Plasma Lipase

- Glycoprotein. $M_r = 48,000$.
- Breakdown of lipids
- Requires ions for its activation ($Na^+$, $Cl^-$)
- Elevated in pancreatic disorders like acute and chronic pancreatitis, pancreatic carcinoma, acute and chronic renal diseases
- Elevation occurs within 2–12 h within the attack (2–4-fold; up to 10-fold).
- Not applied practically.

Plasma Amylase

- Breakdown of complex carbohydrates.
- Normal value: 80–180 Somogyi units
- Alpha (endo) and beta (exo) forms; P and S types
- Activity at pH 6.9–7.0
- Cosmopolitan distribution. Maximum in pancreas
- Useful for the determination of pancreatic disorders
- Elevation (3–6 times) at 2–12 h after attack and returns to normal in 2–3 days
- Non-pancreatic elevations: salivary gland irradiation (S-type), cholecystitis, acute
Cholinesterase

• Hydrolysis of Ach

• 2 types:
  – True cholinesterase (Cholinesterase I)
    • RBCs, lungs, spleen, nerve endings
  – Pseudocholinesterase (Cholinesterase II)
    • Liver, pancreas, heart, white matter of brain, serum

• Important marker for cardiac and liver function
  – Decreased in hepatitis, cirrhosis, carcinoma, chronic renal disease, pregnancy, poisoning
  – Elevated in myocardial infarction (within 3–12 h)
  – Slightly elevated in thyrotoxicosis, obesity, starvation, hyperthyroidism, myxedema, pregnancy, diabetes, stress
Any Questions?

Thank You!

For further resources and queries, contact me

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