Veterinary Clinical Enzymology

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Objectives

1. Introduction to enzymes, briefly.
2. Definition of Clinical Enzymology
3. Knowledge you should know.
4. Enzymes used in clinical diagnosis and their interpretation.
Enzymes

- Enzymes are biological catalysts that effectively increase reaction rates.
- They are proteins(!).
- Substrate specific molecules.
- Partially tissue specific.
- Activity of any enzyme can be determined by measuring the specific reaction rate catalyzed by the enzyme.
Enzyme Activity

- The activity of an enzyme is the expression of the rate of the enzymatic reaction catalyzed by that enzyme by the amount of substrate converted to the product at a certain time under optimal conditions by the action of the enzyme.

- The rate of an enzymatic reaction is related to the activity of the enzyme.

- An enzyme that has more activity transforms more substrate molecules into a product in a given time.

- The most commonly used enzyme activity unit is IU, but the SI unit is katal (kat).
  - 1 katal = 1 mol/second; 1 IU = 1 μmol/min; 1 IU = 16.67 nanokatal
Isoenzymes (or Isozymes)

- Some enzymes have multi-molecular variants that catalyze the same reactions. They are called isozymes/isoenzymes.
  - I.e. ALP, LDH, CK

- They differ in amino acid sequence but catalyze the same chemical reaction.

- Different isoenzymes may be specific to different tissues and thus give an insight into the diagnostic approach.
Clinical Enzymology

Clinical enzymology is the discipline that studies and tests enzyme activity in serum, plasma, urine, or other body fluids for the purpose of helping to establish the diagnosis and prognosis of disease and to screen for abnormal organ function.
Brief History

- **1800**
  - First recognize

- **1908 - 1916 Wohlgemuth**
  - Blood and urine amylase assay

- **1927**
  - Discovery of ALP (Alkaline phosphatase) in bone

- **1950**
  - Development and marketing of simplified enzyme assays in kit form (ALP, ALT, AST)

- **Between 1960 and 1990**
  - Considered as heyday of clinical enzymology
Knowledge you should know

- Location of enzymes.
- For what reasons does the cellular enzymes release from cells to blood?
- What are the properties for enzymes to be used in clinical diagnosis?
- What are the biological materials that may be suitable for measuring enzyme activity?
- How to collect the right biological material?
Biological Materials

- Blood*
- CSF
- Urine
- Seminal Fluid
- Erythrocytes
- Tissue samples etc..

Source: EclinPath
### Routinely Used Enzymes in Clinical Diagnosis

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**ALT (GPT, ALAT)**

- **Alanin aminotransferase/glutamate-pyruvate transaminase**
  - It is an important enzyme in amino acid synthesis and degradation.
  - \( \text{L-glutamata} + \text{pyruvate} \rightleftharpoons \alpha\text{-ketoglutarate} + \text{L-alanine} \)
  - It is a cytosolic enzyme.
  - ALT activity is found in several body organs such as Liver, heart, kidneys, skeletal muscles.
AST (GOT, ASAT)

- **Aspartate aminotransferase/glutamic oxaloacetic transaminase**
  - It is an important enzyme in amino acid synthesis and degradation.
  - Aspartate (Asp) + α-ketoglutarate $\leftrightarrow$ oxaloacetate + glutamate (Glu)
  - It is a cytosolic and mitochondrial enzyme.
  - Found in liver, skeletal and cardiac muscle. It varies between species.
Increased activity of ALT and AST

- If increase in ALT >>> AST?
- If increase in AST >>> ALT?

- AST and other enzymes?
**Sorbitol dehydrogenase**

- **Sorbitol** + NAD\(^+\) $\rightarrow$ **Fructose** + NADH + H\(^+\)
- The active sites of SDH contain Zn\(^{++}\).
- It is located in the cytoplasm of cells.
- The highest concentration of SDH activity is in **liver** followed by kidney, but it is also found in most other tissues at much lower amounts.
- Half-life?
- **Diagnostic value?**
Glutamate dehydrogenase (also known as iditol dehydrogenase)

- It catalyzes the removal of hydrogen from glutamate to form α-ketoglutarate.
- It is a mitochondrial enzyme.
- It is a zinc-containing enzyme.
- The liver has by far the highest concentration of GLDH activity.
- Diagnostic value?
**Alkaline phosphatase (ALKP)**

- It is localized on the cell membrane. It plays a role in membrane transport.
- They have been shown to hydrolyze a range of monophosphates or pyrophosphates at alkaline pH. It is a phosphatase and hydrolyse.
- **There are isoenzymes.**
  - Liver, bone, kidney, adrenal gland, placenta, intestine.
- Cholestasis, CANCER!
- Difference between adolescent and adult?
GGT

- **Gamma Glutamyltransferase/Transpeptidase**
  - It catalyzes the transfer of gamma glutamyl groups from gamma glutamyl peptides such as the tripeptide glutathione to other peptides, amino acids, and water.
  - GGT is a **membrane-bound enzyme** on the external surface of cells.
  - **In liver, GGT activity is primarily associated with the biliary epithelial cells.**
  - It is **more specific than ALP** because it is not found in bone and placenta.
  - Calf?
**LDH**

- **Lactate dehydrogenase**
  - It is located in cytoplasm. The pattern of LDH isozymes present in blood is particularly useful in distinguishing, for example, between myocardial infarction and liver disease (such as infective hepatitis).
  - Two different genes are known to code for LDH. One codes for the M or muscle form, and the other for the H or heart form. There are four subunits in LDH, thus giving five possible isozymes.
  - **LDH;** LDH-5 (M₄), LDH-4 (M₃H), LDH-3 (M₂H₂), LDH-2 (MH₃), LDH-1 (H₄)
    - Skeletal muscle contains some of all five isoenzymes (but predominantly M₄), and heart has predominantly H₄
    - LDH isoenzymes are also present in the liver, kidneys and RBCs.
    - The H₄ isoenzyme is strongly inhibited by pyruvate; however, the M₄ species is not.
  - Catalyzes the **pyruvate-lactate conversion reaction.**
  - Cancer?
- **Creatine kinase (CPK)**
  - It is a cytoplasmic and mitochondrial enzyme.
  - **Mg** is required for activation.
  - It consists of two subunits, B and M.
  - There are 4 isoenzymes.
    - **CK-BB**: Brain, prostate, stomach
    - **CK-MB**: Cardiac muscle, skeletal muscle
    - **CK-MM**: Skeletal and cardiac
    - **CK-Mt**: Mitochondrial membrane
CK

- **Skeletal muscle**
  - % 100 CK-MM

- **Cardiac muscle**
  - % 3-10 CK-MB

- **Brain**
  - 90 CK-BB, % 10 CK-MM + CK-MB

- **Serum**
  - % 50 CK-MM + % 40 CK-BB + % 10 CK-MB

- **Adolescent/Adult difference?**

- **Exercise?**
Amylase

- The highest activity is found in the pancreas and salivary gland.
  - *Pancreatic Amylase* and *Salivary Amylase***

- It is an metalloenzyme and needs **calcium**.

- It cleaves the $\alpha$-D-1,4-glycan linkage of starge and glycogen.

**Acute pancreatitis?**
- Cat : 532 – 2008 U/L
- Dog : 185 – 700 U/L
Lipase

- It hydrolyzes triglycerides.

- *Pancreatic lipase* binds at the lipid-water interface emulsified in the presence of *bile salts*, *colipase*, and *Ca*.

- It is more specific than amylase in the diagnosis of pancreatic damage.

**Diagnostic value?**

- **Total Lipaz Aktivitesi Vs. PLI**
  - Köpek: 78 - 765 U/L
  - Kedi: 5 - 222 U/L
Trypsin

- Its inactive form is trypsinogen (proenzyme).
- It is a *serine protease enzyme*. It cleaves peptide bonds.
- It hydrolyzes proteins.
- It is more specific than amylase in the diagnosis of pancreatic injury.
- TLI (Trypsin-Like Immunoreactivity)
Aldolase

- **Fructose-bisphosphate aldolase**
- It is found in the liver, skeletal-heart muscle and brain which have the highest level of glycolytic activity and energy requirement.

- **Isoenzymes:** ALDO A/B/C

- **Diagnostic value?**
  - Skeletal muscle injury and liver damage
  - Compare to CK
  - Investigation of root cause of muscle weakness
Other enzymes

- GPx (*Glutathione peroxidase*)
- SOD (*Superoxide dismutase*)
- Acid phosphatase
- **Pseudocholinesterase/Butyrylcholinesterase**
  - Read from [Biyokimya.vet](http://biyokimya.vet)
- **5’ Nücleotidase**
  - Hepatobiliary system diseases
- G-6-PD
Measurement of Enzyme Activities

- End-Point Methods
- **Kinetic Methods**
- Electrophoretic Methods
- Isoelectric Focusing
- Immunohistochemistry
Your Questions?

Send to serkan.sayiner@neu.edu.tr
References

Next topic; Liver Function Tests
For more on Biochemistry & Clinical Biochemistry and the world of laboratories follow

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