UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DISCIPLINE OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
BMLS & BDS Year 3
CLINICAL RELEVANCE OF ENZYMOLOGY – An Overview

What are the enzymes that are present in blood plasma?

- Enzymes are found in all tissues, as well as in blood plasma
- Enzymes in plasma can be separated into two groups:

- **Functional Plasma Enzymes**:
    - Enzymes that are specific to plasma and have Functional role
    - Enzymes that are synthesized in Liver but present in blood in equivalent or higher concentrations than in tissues

    - **Examples of Functional plasma enzymes**:
        - Lipoprotein Lipase – involved in processing of Chylomicrons (Lipid metabolism),
        - Pro-enzymes of blood coagulation (formation of Thrombin) and blood clot dissolution (Plasmin),
        - Pseudocholinesterase, etc.

- **Non-Functional Plasma Enzymes**:
    - Enzymes that do not perform any know functions in plasma
        - Substrates for these enzymes are usually not present in Plasma
        - Plasma levels of these enzymes are several times lower than in tissues
    - Damage to tissues usually result in significant increase in the level of these enzymes in plasma
    - Duration of these enzymes in plasma depends on rate of Clearing, which is usually determined by:
        - Stability of the Enzyme and
        - Susceptibility to Reticuloendothelial system

- **Examples of Non-Functional Enzymes**:
- **Exocrine Enzymes**: such as
    - Pancreatic Amylase, Lipase, Bile Alkaline Phosphatase, Prostatic Acid Phosphatase etc.

- **True Intracellular Enzymes**:
    - Most of these enzymes are located in Specific Organelles in the cell thus there appearance in plasma varies

  Examples:
    - Cytoplasmic enzymes will appear in plasma before Mitochondrial enzymes

What causes enzymes to be release into plasma?

- Concentrations of enzymes in cells are relatively higher than in plasma
- Some causes for release of enzymes into plasma include:
  - Cellular damage resulting from disease or trauma
  - Increased rate of cell turnover during active growth,
  - Tissue repair, Cancer
  - Increased concentration of enzymes within cells, usually as a result of induction by disease or drugs
  - Duct obstruction, etc

Why are some specific enzymes selected for use in clinical diagnosis?

- Some important diagnostic questions for selecting specific enzymes include the following:
  - If it is suspected that tissue damage has occurred then
    - **What is the extent of the damage**?
      - Sensitivity of the enzyme test(s) becomes the determining factor,
        - This mainly depends on the ratio of the enzyme activity in the Tissue and Plasma
        - For most enzymes the Tissue to Plasma Ratio is between 1000 to 1 and 10000 to 1
  - **In which tissue has the damage occurred if any**?
    - Since only few enzymes are Tissue-Specific, this is usually a major problem
      - In most cases, the problem can be overcome by either selecting Isoenzymes or Combination of Enzymes
        - Example: Liver Function Tests (LFT) uses combination of enzymes
  - **During the cause of the disease does the activity of the enzyme in plasma changes, if so how**?
    - This usually depend on Rate of release of the enzyme into Plasma and Rate of Removal from plasma
    - Timing becomes a factor in the diagnosis

SOME ENZYMES OF CLINICAL INTEREST:

PLASMA CHOLINESTERASE (ChE: formerly called PSEUDO-CHOLINESTERASE):

- Plasma Cholinesterase is different from Acetylcholinesterase (formerly called True-Cholinesterase)
- Acetylcholinesterase is in Cholinergic Neurones and in RBC but not in Plasma
- Acetylcholinesterase catalyzes degradation of Acetylcholine
- Plasma Cholinesterase is produced in the liver and release into blood plasma
- Plasma Cholinesterase catalyzes breakdown of Choline Esters in Plasma

- Example: **Succinylcholine** (**Scoline, Suxamethonium**) a muscle relaxant used in Anaesthesia

- Individuals with low levels of Plasma Cholinesterase are unable to metabolise Scoline normally
  - Resulting in prolonged paralysis after Anaesthesia
    - A phenomenon called "**Scoline Apnoea**"

- To prevent "Scoline Apnoea" determine the Plasma Level of Cholinesterase before exposing patients to this group of Anesthetics

What parameter is used to assess Cholinesterase in blood plasma?

- **Dibucaine Number** (**DN**) is used to assess plasma level of Cholinesterase
  - Dibucaine is a compound that Inhibit Plasma Cholinesterase
- DN is an indication of the **Type** of Plasma Cholinesterase present in the blood of an Individual
- DN and Plasma Cholinesterase level of an individual can be used as an Index of the extent of exposure to certain Insecticides and Pesticides
- **To assess the dose of Succinylcholine and other similar Acetylcholine-like Anesthetic drugs to be administered to a patient, one needs to know the DIBUCAINE NUMBER (DN) of the patient**

How can Dibucaine Number of a patient be determined?

- Substrate for Plasma Cholinesterase determination is Benzoy1choline
- Determination of Dibucaine number requires the estimation of the activity of Plasma Cholinesterase, using Benzoylcholine as Substrate and 0.01mM Dibucaine as Inhibitor
  - % Inhibition of Plasma Cholinesterase by Dibucaine is then calculated
- Percentage Inhibition of Plasma Cholinesterase by Dibucaine represents the Dibucaine Number
- Dibucaine inhibits "Normal" Plasma Cholinesterase by 80%
  - Therefore, Normal Dibucaine Number (DN) is 80
- An individual with DN of about 20 can suffer severe Scoline Apnoea

What are some of the causes of Low Plasma Cholinesterase?

- Inherited abnormality:
  - Most of individuals that are sensitive to Scoline may have Genetically determined abnormalities resulting in Low Plasma Cholinesterase
- Liver disease:
  - Causes impaired protein synthesis, which may cause Low Plasma Cholinesterase
- Industrial Poisoning:
  - Organo-phosphorus Insecticides or Pesticides
- Drug effects:
  - Oral Contraceptives, Monoamine Oxidase Inhibitors, Cytotoxic drugs, etc

URINARY ENZYMES (ENZYMURIA):

What are the advantages of Enzymuria?

- Sensitivity, Reliability and Specificity of Urinary Enzyme measurement as Routine, Non-Invasive and Reliable Test for Evaluation of Nephropathy and Early Detection of Renal Damage caused by Drugs or other substances is well documented

- Valuable information on the mechanism and site of renal damage can be gained from the measurement of Urinary Enzymes that are Specific for different regions of the Nephron or its sub-cellular compartments

- Urinary Enzyme levels are valuable as diagnostic tools during the Early Stages of Renal Diseases and quite often measurements also provides information on the progress of renal disease and possible reversibility of the disease process

- **No single urinary enzyme assay** can be proposed as an indicator of renal damage, only a combination of two or more urinary enzymes can serve as useful screening tools for renal diseases and provide an early warning signal of renal injury

- Changes in some urinary enzyme levels precede and predict the subsequent development of clinically overt nephropathy in diabetes mellitus

- Enzymuria and Proteinuria may be of value to the Clinician looking for signs of early renal damage in patients on Anti-hypertensive therapy when blood pressure control is judged to be adequate

- Challenge that urinary enzyme assay posses to the Clinician are how to incorporate these new data derived from a highly sensitive, non-invasive test with the more familiar and long established but less sensitive functional tests used for assessing the functional state of the kidney.

- Enzymuria is more sensitive than the Classical Renal Function Tests (Serum Creatinine, Serum Urea, Creatinine or Inulin Clearance, Urinalysis, Osmolality and Electrolyte in urine)

    - Classical Renal Function Tests: Levels of Parameters estimated are elevated above normal at a later stage and usually after damage to the Kidney have progressed

What are the Urinary Enzymes of interest in diagnosis?

Urinary enzymes and other parameters of interest are as follows:

- **Alanine Aminopeptidase (AAP, EC. 3.4.11.2);**
- **Gamma Glutamyl Transferase (GGT, EC. 2.3.2.2);**
- **N-Acetyl-B-D-Glucosaminidase (NAG, EC. 3.2.1.30);**
- **Alkaline Phosphatase (ALP, EC. 3.1.3.1);**
- **Lysozyme (Muramedase) (MUR, EC. 3.2.1.17);**

- **Lactate Dehydrogenase (LDH, EC. 1.1.1.27);**
- Creatinine; Protein and Urine Flow Rate.

ALKALINE PHOSPHATASE (ALP):

- Catalyses the removal of Phosphate groups at Alkaline pH.
- Widely distributed in tissues, High concentrations in Intestines, Liver, Bone, Spleen, Placenta and Kidney
- Useful in diagnosis of Hepatobiliary (Cholestatic liver disease) and Bone disorders
- Isoenzymes of ALP are used to distinguish between Liver and Bone diseases
- Isoenzymes are most easily differentiated by Heat Stability Test and by Electrophoresis
    - Liver ALP Isoenzyme (ALP1) is Heat Stable
    - Bone ALP Isoenzyme (ALP2) is Inactivated by Heat
- Detection of Isoenzymes help differentiate the source of the Pathologic condition associated with Elevated Total ALP

GAMMA GLUTAMYL TRANSPEPTIDASE (GGTP OR γGT):

- Catalyses transfer of Amino Acids and Peptides across Cellular membrane and is involved in **Glutathione metabolism**
- Highest concentrations found in Liver and Biliary Tract
- Lesser concentrations in Kidney, Spleen, Heart, Intestine, Brain, Prostate
- GGTP levels higher male because of additional levels in Prostate
- GGTP is used to detect liver dysfunction and Cholestasis
- As with 5'Nucleotidase, elevation of GGTP generally parallels that of ALP however, GGTP is more sensitive
- Unlike ALP, GGTP and 5'Nucleotidase are not increased in bone disease
- Normal GGTP with Elevated ALP indicates Bone disease
- Elevated GGTP and Elevated ALP indicates Hepatobiliary disease
- Unlike ALP, GGTP is not elevated in Childhood or Pregnancy
- GGTP can be used to detect Chronic Alcohol Ingestion
- GGTP is very useful in screening and evaluation of Alcoholic Patients
- GGTP is elevated in about 75% of patients who chronically drink alcohol

ACID PHOSPHATASE (ACP):

- Catalyses removal of Phosphate groups at Acid pH
- Found in Prostate, Bone, Liver, Spleen, Kidney, RBC, Platelets
- Used to diagnose Prostate dysfunction

ALANINE AMINOTRANSFERASE (ALT, old name is SGPT):

- Widely distributed, but high concentrations are found in liver
- Useful in diagnosis of Hepatocellular damage (LFT)

ASPARTATE AMINOTRANSFERASE (AST, old name is SGOT):

- Widely distributed, high concentrations in Cardiac muscle, Skeletal muscle, and Liver
- Useful in diagnosis of MI, Hepatocellular Damage and Muscle damage

CREATINE KINASE (CK):

- Creatine Kinase (Creatine Phosphotransferase)

- Catalyzes formation of Creatine Phosphate

ATP + Creatine ⟵=====⟶ Creatine Phosphate + ADP

- CK occurs as a dimer of two different subunits, M and B
- Isoenzyme types are:
    - CK-BB: Brain type; CK-MB: Hybrid type; CK-MM: Muscle type

- Isoenzymes can be separated by Electrophoresis
- Elevated blood levels of Total CK are used as indicators of MI, muscular dystrophy, and Stroke
- CK-MB is released from Cardiac muscle cells after Myocardial Infarction

GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G6PD):

- Catalyses first reaction in Pentose Phosphate Pathway (Hexose Monophosphate shunt; HMP shunt)
- Important in production of NADPH + H^+ especially in the RBC
- NADPH + H^+ is use to maintain normal level of Reduced Glutathione (GSH) in RBC
- Individuals with G6PD deficiency:
    - If given certain antimalarial drugs or Oxidants may develop Haemolytic Anaemia, Cyanosis, Jaundice

LACTATE DEHYDROGENASE (LDH or LD):

- LDH catalyses reversible conversion of Pyruvate to Lactate under Anaerobic conditions

Pyruvate + NADH + H^+ ⟵====⟶ Lactic Acid + NAD

- LDH occurs as Tetramer of Two different Subunits, thus forming Five Isoenzymes:
    - LD-1 (HHHH) from Cardiac Muscle: Elevated after MI
    - LD-2 (HHHM) from Kidney: Elevated after Renal Infarction
    - LD-3 (HHMM) from Lung, Spleen, Pancreas: Elevated in Pulmonary Embolism
    - LD-4 (HMMM) and LD-5 (MMMM), both from Liver and Skeletal Muscle: Elevated in Injury to Liver or Skeletal Muscle

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