Biochemical markers for diagnosis of diseases and follow up

Foundation Block

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Lecture objectives

Upon completion of this lecture, the students should be able to:

- Comprehend the importance and diagnostic qualities of various biomarkers
- Understand the importance of different biomarkers in the diagnosis, treatment and follow up of a disease.
- Recognize the types of biomarkers and their use in specific diseases such as heart, cancer, liver, kidney and pancreatic diseases.

Overview

- What is a biomarker?
- Diagnosis and prognosis
- Plasma and tissue-specific biomarkers
- Factors affecting serum biomarker levels
- Qualities of a good biomarker
- Types: Enzymes, proteins, hormones
- Enzymes: Amylase, lipase, trypsinogen, ALT, AST
- Proteins: Cystatin C, BNP, α-fetoprotein, PSA
- Hormones: Anti-Mullerian hormone

What is a biomarker?

- A biological molecule found in blood, other body fluids, or tissues that indicates a normal or abnormal process such as a disease or a condition
- A biomarker is measured to follow up a disease or treatment

diagnosis and prognosis

Diagnosis:

- Identification of a disease from its signs and symptoms

Prognosis:

- The future outcome of a disease

Most common body fluids for measurement of biomarkers are:

- Serum
- Plasma
- Urine

Some biomarkers are either:

- Plasma-specific or
- Tissue-specific

Plasma-specific biomarkers

- Normally present in plasma
- Perform their functions in blood
- High level of activity in plasma than in tissue cells
- Examples: blood clotting enzymes (thrombin), cholinesterase, etc.

Tissue-specific biomarkers

- Present inside the cell
- Conc. is lower in plasma
- Released into the body fluids in high conc. due to:
  - cell damage
  - defective cell membrane

- Intracellular enzymes are present only in their cells of origin
- Some are secretory enzymes that are secreted by salivary glands, gastric mucosa and pancreas
- In disease, plasma levels of secretory enzymes increase when their cells are damaged
- The diagnosis of organ disease is done by measurement of enzymes of that tissue

Factors affecting serum biomarker levels

- Cell damage
- Rate of biomarker synthesis and clearance
- Enzyme inhibitors
- Glucose deficiency
- Localized hypoxia (less oxygen)
- Ischemia (obstruction of blood vessels)
- Necrosis
- Tissue infarction due to ischemic necrosis
- Myocardial infarction

Qualities of a good biomarker

A good biomarker should be:

- Able to accurately diagnose a disease
- Able to accurately predict prognosis of a disease
- Compliant with treatment follow up
- Easily obtainable from blood, urine, etc.

Qualities of a good biomarker assay

- A good biomarker assay should be rapid to deliver results faster
- Sensitive
  - Ability of an assay to detect small quantities of a marker
- Specific
  - Ability of an assay to detect only the marker of interest

Types of biomarkers

- Enzymes
- Hormones
- Proteins

Enzymes as biomarkers

- Enzymes are clinically used for the diagnosis and prognosis of various diseases

Examples include:

- Amylase, Lipase
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

Amylase and Lipase

- Acute pancreatitis is the inflammation of pancreas caused by:
  - Obstruction of the pancreatic duct
  - Gallstones
  - Alcohol abuse
- Abnormal release and premature activation of pancreatic enzymes (for example amylase, lipase)
- Diagnosis conducted by measuring pancreatic enzymes

Amylase

- Elevated serum amylase level is a diagnostic indicator of acute pancreatitis
  - Amylase level greater than 10 times the upper limit indicates acute pancreatitis
- The test has low specificity because elevated serum amylase level is also present in other diseases
- Amylase appears in the serum within 2-12 hours after abdominal pain
- Free amylase (unbound form) is rapidly cleared by the kidneys

Lipase

- Serum lipase has higher specificity than serum amylase
- It appears in plasma within 4-8 hours and remains for 8-14 days
- Measurement of amylase and lipase give 90-95% accuracy in the diagnosis of acute pancreatitis and abdominal pain

High serum ALT and AST levels in liver diseases are due to:

- Alcohol abuse
- Medication
- Chronic hepatitis B and C
- Steatosis and steatohepatitis
- Autoimmune hepatitis
- Wilson's disease
- α_1-antitrypsin deficiency
- Malignancy
- Poisons and infectious agents

Alanine aminotransferase (ALT)

- Mostly present in liver
- Small amounts in heart
- More specific for liver disease than AST
- Major diagnosis: liver disease

Aspartate aminotransferase (AST)

- Widely distributed in heart, liver, skeletal muscle, kidney
- Small amounts in erythrocytes
- High serum activity of AST found in:
  - Liver disease, heart disease, skeletal muscle disease, hemolysis
- Major diagnosis: liver and muscle diseases

Serum enzymes used in the assessment of liver function:

- Markers used in hepatocellular necrosis
  - Alanine aminotransferases
  - Aspartate aminotransferases

Proteins as biomarkers

- Cystatin C
- B-type natriuretic peptide (BNP)

Tumor markers

- α-Fetoprotein
- Prostate Specific Antigen (PSA)

Cystatin C

- A cysteine protease inhibitor mainly produced by all nucleated cells of the body
- Useful biomarker for measuring glomerular filtration rate (GFR) in assessing kidney function and failure
- Unlike creatinine, its serum conc. is independent of gender, age or muscle mass
- Abnormally high serum levels of cystatin C indicates kidney failure
- Clinically useful marker for detecting:
  - early kidney disease, monitoring kidney transplantation and acute kidney injury

B-type natriuretic peptide (BNP)

- A peptide secreted mainly in the cardiac ventricles in response to cardiac expansion and pressure overload
- High serum levels are observed in congestive heart failure
- In some pulmonary diseases, BNP levels are high but not as high as in heart failure
- BNP helps differentiate between pulmonary disease and heart failure
- An important marker for the diagnosis and prognosis of congestive heart failure
- Currently being investigated as a screening biomarker for heart disease

Tumor markers

- A molecule secreted by a tumor that is measured for diagnosis and management of a tumor

- α-fetoprotein
- Prostate specific antigen (PSA)

α-Fetoprotein

- In newborn babies α-fetoprotein levels are very low
- High conc. are observed in:
  - Hepatocellular carcinomas (hepatoma)
  - Testicular carcinomas
  - GI tract carcinomas
- However, high serum levels are also found in benign (non-cancerous) conditions e.g. hepatitis
- High conc. are not always suggestive of a tumor

Prostate Specific Antigen (PSA)

- A serine protease enzyme also called kallikrein III, seminin
- Produced by prostate gland
- Liquefies ejaculate
- High serum PSA levels are observed in prostate cancer
- Less specific in diagnosis
  - High serum levels are also observed in benign prostatic hypertrophy (enlarged prostate gland)

Hormones as biomarkers

Anti-Mullerian hormone (AMH)

- A polypeptide hormone involved in sexual differentiation of male embryo
- In females it is produced by ovaries
- Prevents premature depletion of ovarian follicles
- Appears to be a best marker for estimating egg cell reserve in the ovaries
- Only growing follicles produce AMH
- Plasma AMH levels strongly correlate with number of growing follicles
- Helps assess female fertility

Take-home messages

- Biomarkers are used for diagnosis, prognosis and follow up of diseases
- A biomarker should exhibit good diagnostic and prognostic values
- Examples of biomarkers used in different disease will help understand their qualities and limitations

References

- What are biomarkers? Kyle Strimbu and Jorge A. Tavel, *Curr Opin HIV AIDS*. 2010 November ; 5 (6): 463–466
- Biomarkers: Potential uses and limitations. Richard Mayeux. *J. Amer. Soc. Exp. Neuro Therap*. Vol. 1, 182–188, April 2004