Review Paper

CLINICAL DIAGNOSIS of DISEASE STATES USING ENZYMES AND PROTEINS (REVIEW)

ABSTRACT

Disease states usually lead to moderate or extensive tissue damage depending on the time of onset and severity of the disease. Such tissue damages are usually associated with the release of enzymes (specific to the diseased organ or tissue) into circulation which results in an increase in activity of such enzymes in body fluids. The measurement of these changes in enzymatic activity is usually employed as an important clinical assessment tool for detecting, diagnosing and monitoring diseases and pathological processes. Some of the enzymes used in diagnosis include transaminases (in liver diseases), creatine kinase (in myocardial infarction), amylase (in pancreatitis), acid phosphatase (in malignant diseases), and alkaline phosphatase (in bone diseases). Some other enzymes are used as diagnostic reagents in detecting the presence of compounds of clinical importance. These include glucose oxidase (for detecting the presence of glucose), urate oxidase (for testing the presence of uric acid) and cholesterol oxidase (for testing the presence of cholesterol) in diabetes, kidney stones and arteriosclerosis respectively. Various body fluids also contain proteins other than enzymes that are of diagnostic importance especially the plasma proteins. The plasma proteins are broadly divided into two namely; albumin and globulin. The globulins include gamma-globulins, beta-globulins, alpha-1 globulins and alpha-2 globulin. Many physiological and/or disease conditions produce changes in these individual plasma protein concentrations, and measurements of these changes can provide diagnostic information. Some of such enzymes and proteins of diagnostic importance are discussed in this review.

Keywords: Phosphatase; Transaminases; Arteriosclrosis; Albumin; Oxidase

INTRODUCTION

Enzymes are soluble, colloidal organic catalysts synthesized by living cells [11]. The large numbers of enzymes present in the human body are synthesized intracellular, and for most, their functions are also exercised within the cells that produced them. Some are however; secreted into the intracellular fluids (e.g blood) and they can be further divided into two classes namely; 1) Functional plasma enzymes and 2) Non-functional plasma enzymes [20]. Functional plasma enzymes also known as plasma specific enzymes are present at all times in the circulation of normal individuals and perform specific physiologic functions in the blood. The functional enzymes include lipoprotein lipase, pseudocholinesterase and pro-enzymes of blood coagulation and fibrinolysis [25]. The second class known as non-functional plasma enzymes (cell- derived

enzymes) perform no function as such in the blood but are present in the circulation as a result of the normal wear and tear processes of the cells. The non-functional enzymes include the transaminases (Alanine aminotransferase and Aspartate aminotransferase), lactate dehydrogenase enzyme and alkaline phosphatase [25].

Disease states usually lead to moderate or extensive tissue damage (depending on the time of onset and severity of the disease) which eventually leads to the release of enzymes (non-functional enzymes specific to the diseased organ or tissue) into circulation resulting in an increase in the activity of these enzymes in body fluids [6]. The basic principle of using enzyme levels for diagnosing disease is based on comparing the changes in activity in serum or plasma of these enzymes which are usually present in the serum in very low active amounts under normal circumstances [2]. A sensitive analysis would give insight into the pathological changes and nature of the disease. However, as the enzymes and their isoforms may belong to varied tissue types, it is of significant relevance to have a detailed knowledge of isoenzymes of the enzymes understudy and their enzymatic properties like kinetics, effect of factors like temperature and pH, rate of release from the cells of origin and rate of clearance from circulation [2].

Various body fluids also contain proteins other than enzymes that are of diagnostic importance especially the plasma proteins. The plasma proteins are numerous and are varied in their origin and functions. Albumin is regarded as the single most important quantitative plasma protein. Most of the other plasma proteins are collectively grouped as globulins. Many physiological and/or disease conditions produce changes in individual plasma protein concentrations, and measurements of these changes often provides diagnostic information [20].

The relevance of enzymes and plasma proteins in clinical diagnosis of diseases cannot be over emphasized. Thus, this write up highlights some of such useful enzymes and proteins.

ENZYMES IN HEPATO-BILIARY DISEASES

Alanine transaminase (ALT)

ALT was formally known as Glutamic Pyruvate Transaminase (GPT). It catalyses the reversible transamination of L- alanine and 2- oxoglutarate to pyruvate and glutamate in the cytoplasm of the cell, it can be found in the liver, skeletal muscle and heart. ALT Increased serum level of

ALT indicates a severe liver disease, usually viral hepatitis and toxic liver necrosis. Kim *et al.* (2004) reported that ALT is a common serum marker of liver disease. Even a minor elevation of ALT is a good indicator of severity in liver disease.

Aspartate transaminase (AST)

AST, also known as serum glutamate oxaloacetate transaminase (SGOT), is a pyridoxal phosphate (PLP) dependent enzyme that catalyses the reversible transamination of L- aspartate and 2- oxoglutarate to oxaloacetate and glutamate Significant increase in the serum level (10-100 times the normal (0- 40 IU/L) of AST indicates severe damage to liver (viral hepatitis or toxic liver necrosis) or heart cells (MI) [15]. AST could be a useful marker to screen liver fibrosis.

Alkaline phosphatase (ALP)

The increase in the level of serum ALP indicates an increased hepatocytic activity in hepatobiliary disease. Higher ALP levels in serum are observed when bile ducts are blocked as in the case of cholestasis [3].

Gamma glutamyl transferase (GGT)

Gamma-glutamyltransferase (GGT) is an enzyme that transports amino acids; it is present in the cell membrane of nearly all human cells. This enzyme is sometimes referred to as a "transpeptidase". Specifically, it catalyzes the transfer of a gamma glutamyl group to another acceptor. It is most abundant in the kidney, liver, pancreas and intestine, but the majority of the GGT detected in serum derives from the liver. GGT is the most sensitive biomarker of hepatobiliary disease [21]. Increases occur earlier and persist longer than ALP in cholestatic disorders [26].

ENZYMES IN MYOCARDIAL INFARCTION

Creatine kinase- MB (CK- MB)

The death of the heart muscle due to myocardial infarction (MI) prompts the release of several molecules such as creatine kinase (CK) into the circulation. Khan *et al*. (2012) reported in an experiment that serum CK levels are significantly higher in patients with acute infarction than

that of control (normal range: 10- 50 IU/L). Three isoforms of CK exists namely: MM, MB and BB isoforms. CK-MB which is the isoform present in the heart is the most specific and accurate means of detecting MI than total CK estimation [24].

Other useful markers in MI are myoglobin, troponins Aspartate transaminase (AST) and Lactate dehydrogenase (LDH) [3, 10].

ENZYMES IN MALIGNANT DISEASES

Acid phosphatase (ACP)

Five important isoforms of ACPs exists. They are the lysosomal, prostatic, erythrocytic, macrophage and osteoclastic forms [2]. They differ widely with tissue and chromosomal origin, molecular weight, amino acid homology, sequence length, and resistance to L (+) tartrate and fluoride [1]. ACP level in male prostate gland is 100 times more than in any other body tissue. Kirschenbaum *et al.* (2011) have reported that prostatic acid phosphatase (PAP) is strongly expressed by prostate cancer cells, especially in bone metastases.

ENZYMES IN MUSCULAR DISEASES

The most commonly measured and most reliable and sensitive biochemical index of muscle diseases is creatine kinase (CK) measurement. Both AST and Aldolase are also useful indices but are less sensitive. CK is high in muscular dystrophies, polymyositis as well as toxic myopathies [7].

ENZYMES AS DIAGNOSTIC REAGENTS

Some enzymes are used as reagents to detect the presence of compounds of clinical importance. Below are examples of such enzymes

Enzymes	Compounds Detected	Disorder	References
Urease	Urea	Renal diseases	De Melo *et al.,* 2002
Oxalate oxidase	oxalate	Kidney stones	Reddy and Vadgama, 1997

Glucose oxidase	Glucose	Diabetes	Wang *et al.,* 2011
Cholesterol oxidase	Cholesterol	Arteriosclerosis	Marazuela *et al.,* 1997
Glutamate	Glutamate oxidase	Neuropathy	MacLamore *et al.,* 2010
Acetylcholinesterase	Acetylcholine	Neurological problems	Horiuchi *et al.,* 1997
Lactate oxidase	Lactate	Ischaemic myocardium	Marzouk *et al.,* 1997

PLASMA PROTEINS IN DIAGNOSIS

Proteins are the most abundant compounds in human serum. The major measured serum proteins are divided into two groups namely: Albumin and Globulins.

A typical blood panel will provide four different measurements namely: (i) Total protein (TP) (ii) Albumin (iii) Globulins and iv) Albumin- Globulin Ratio [28].

Total Protein (TP)

The total protein represents the sum of albumin and globulins. Ideally, the total protein is approximately 7.5 g/dl and optimal range of about 7.2- 8.0 g/100ml. The total protein may be elevated due to chronic infection, adrenal cortical hypofunction, liver dysfunction, collagen vascular disease, hypersensitivity states, dehydration and respiratory distress while it could be decreased due to malnutrition and malabsorbtion, liver diseases, diarrheoa, pregnancy etc [5].

Albumin

Albumin is synthesized in the liver. Its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. A very strong predictor of health; low albumin is a sign of poor health. Its optimal range is 4.5- 5.0 g/100ml. albumin levels may be elevated in dehydration, poor protein utilization, congestive heart failure and may be decreased in malnutrition, polydipsia, and liver dysfunction [8].

Globulins

Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. The specific profile of the globulins is determined by protein electrophoresis, which separates the proteins according to size and charge [17].

There are four major groups that can be identified: gamma globulins, beta globulins, alpha-2 globulins and alpha-1 globulins. Once the abnormal one has been identified, further studies can determine the specific protein excess or deficit [23].

Albumin/ Globulin Ratio

The liver can function adequately on 20% of liver tissue, thus early diagnosis by laboratory methods is difficult. A reversed A/G ratio may be a helpful indicator. The optimal range is 1.7-2.2. The AG ratio may be elevated in hypothyroidism, hypogammaglobulinemia and could be decreased in liver dysfunction [17].

CONCLUSION

Enzymes and plasma proteins play a pivotal role in clinical diagnosis. Enzymes have a wide range of applicability from diagnostic markers to diagnostic reagents as a result of their high specificity. Though there are a large number of enzymes and proteins in diagnostic use already, more research has to be focused on elucidating the potentials of more enzymes to aid the diagnosis of the numerous diseases of man.

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