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ISO-ENZYMES IN DIAGNOSTICS: A REVIEW

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ABSTRACT

New areas of problems keep surfacing as a result of the complex interactions among the various factors which eventually lead to diseases. Extensive studies of these different factors abnormality under taken so far have been able to provide an insight into the patho-physiological alterations in various diseases. Physiologically, iso-enzymes are structurally different forms of the same enzyme and perform the same enzyme activity. In this review, we tried to provide an overview about the various forms, structure and functions of different iso-enzymes with special focus on their tissue localization.

KEYWORDS: Hepato-biliary diseases, Cancer, Leucocyte, Prostate gland.

INTRODUCTION

New areas of problems keep surfacing as a result of the complex interactions among the various factors which eventually lead to diseases. Extensive studies of the different factors abnormality under taken so far have been able to provide an insight into the patho-physiological alterations in various diseases.[1]

Physiologically, iso-enzymes are structurally different forms of the same enzyme and perform the same enzyme activity.[2,3] The first research according to internet on the iso-enzymes dated as far as back to 1957, where a report mentioned iso-enzymes are the different variants of same enzymes having similar function in the same individual.[4] Later on, curiosity in the role of iso-enzymes in medical research has been investigated. An intensive search over the literature through the years reported that iso-enzymes are formed in different ways, attributing to presence of origin from the same locus of the gene and post translational molecular heterogeneity of enzyme protein synthesized.[5-8] Further searches on the identification of iso-enzymes revealed that they are separated by electrophoresis, heat stability, by their inhibitors, by substrate specificity, by co-factor requirements, antibodies specificity, and lastly their tissue localization.[9-15] However, there is a lot more research to be done for coming to a clear conclusion about the formation of iso-enzymes and their association with different diseases. In this review, we tried to provide an overview about the various forms, structure and functions of different iso-enzymes including adenosine deaminase (EC 3.5.4.4), aldolase (EC 4.1.2.13), alkaline phosphatase (EC 3.1.3.1), beta-glucuronidase (EC 3.2.1.31), creatine kinase (EC 2.7.3.2), enolase (EC 4.2.1.11), glucose-6-phosphatase (EC 3.1.3.9), lactate dehydrogenase (EC 1.1.1.27), and prostate specific antigen (EC 3.4.21.77).

Adenosine Deaminase (ADA)

ADA is an enzyme that converts adenosine to inosine, and deoxyadenosine to deoxyinosine.[16] It is best known in the medical science for its role in cell-mediated immunity and helps in insulin activity.[17] Research reported low levels of ADA are observed in individuals with malnutrition and patients with human immunodeficiency virus.[18] Higher values of ADA are reported in diseases including brucellosis, leprosy, infective mononucleosis, viral hepatitis, and liver cirrhosis.[16-19] Till now literature has demonstrated two isoforms of ADA present in human individuals, ADA1 and ADA2. ADA1 is found mainly in lymphocytes and macrophages.[20] The two isoforms are interconverted to each other in the lung. ADA2 was also found in macrophage and co-exists with ADA1.[21] Reports have

been found that ADA2 iso-enzyme activity is of considerable prognostic value in AIDS and adult T-cell leukemia (ATL) cases.[18-22]

Aldolase

Aldolase is an enzyme that helps convert glucose (sugar) into energy and exists in three isoforms, Aldolase A, Aldolase B and Aldolase C.[23] These are found throughout the body. Aldolase A converts fructose 1,6 Bisphosphate to glyceraldehydes 3 phosphate and dihydroxyacetone phosphate, present in liver kidney and intestine.[24] Reports suggested that Aldolase-A deficiency is attributed to myopathy and hemolytic anemia.[23-25] Aldolase-B helps in fructolysis that is by converting fructose-1-phospahte to glyceraldehydes and dihydroxyacetone phosphate present preferentially in liver.[25] Deficiency of this iso-enzyme leads to a condition called as hereditary fructose intolerance. Aldolase-C helps in aldose cleavage and present abundantly in brain and nervous tissue.[26] In a report by Asaka et al[25], patients affected with muscle abnormalities and cancer found increased levels of aldolase A, but not increased in the patients affected with liver diseases. On the contrary in the same report, patients affected with liver diseases found increased levels of aldolase B, but not increased in the patients affected with muscle abnormalities and cancer patients. Further, the report observed a decrease in serum aldolase B levels in cancer patients. In another report by Ojika et al [26], all the three iso-enzymes of aldolase were estimated in tumor tissues and sera of patients with lung cancer. In patients affected with lung cancer, the report observed aldolase A and aldolase C tissue levels were increased but not aldolase B tissue levels. However, no increase in the sera of these iso-enzymes were observed in the sera of patients with lung cancer.

Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is a glycoprotein that removes the phosphate group from various proteins and nucleotides at basic pH values.[27] ALP is divided into six iso-enzymes depending upon the site of tissue expression that are Alpha-1 ALP, Alpha-2 heat labile ALP, Alpha-2 heat stable ALP (Regan), pre beta ALP, gamma ALP, the leucocyte ALP.[5] From the accumulated research it is believed that ALP iso-enzymes originated from the duplications of primordial tissue non-specific ALP (TN-ALP) gene into six different forms.[28] Reports have also been demonstrated that alteration in the handling of a specific gene (TN-ALP gene) product during and after its formation gives rise to specific isoforms of ALP enzymes.[28-30] These modifications include amount of carbohydrate content attached to different iso-enzymes, thus differing iso-enzymes between each other.[28] Further research has also revealed that only humans and our ancestral origin have placental ALP.[30]

Individuals affected with hepato-biliary diseases including cholestasis or hepatic carcinoma have very high levels of ALP.[31-33] Increase in the same isoform is noticed in the patients affected with bile duct obstruction.[31] Slight increase in the ALP is observed in the hepatic tissue diseases, however in patients affected with hepatitis, and also in patients where inflammatory edema produces an obstructive phase, increase in ALP is seen.[32] In patients affected with gall stones or in patients where bile duct is obstructed by the cancer of head of pancreas or in patients suffering with intra-hepatic obstruction due to drugs observe very high levels of ALP.[32,33] In patients affected with bone diseases or cancers of bone or in hyperparathyroidism observe very high levels of ALP.[34,35] Placental origin ALP can be easily identified if an iso-enzyme of ALP is inhibited by phenylalanine and such iso-enzyme increase is seen normal pregnancy.[36] An iso-enzyme of ALP similar to placental ALP is characteristically seen in circulation about 15% cases of carcinoma of pulmonary tissue, hepatic tissue and digestive tissue and named as Regan iso-enzyme or carcinoplacental iso-enzyme.[37]

Utmost care has to be inducted into interpretation of ALP concentration in the laboratory as there is an intestinal form of ALP where the same can alter the actual concentration of ALP during fasting or anorexia or in postprandial.[30, 32-37]

Beta-Glucuronidase (β-Glu)

Beta-Glucuronidase enzyme (β-Glu) is a lysosomal hydrolase involved in the stepwise catabolism of glucuronic acid-containing glycosaminoglycans.[38] The first report on this isozyme was reported in the year 1965.[39] Despite the role of β-Glu has been studied for several years, the physiological role is still remains obscure. Nevertheless, three isoforms have been identified depending on the pH of its surrounding medium. Fondo et al[40], found three peaks of activity at pH 4.5; pH 5.2; pH 6.1-6.3, respectively in normal premenopausal female mammary gland and lung tissues the report further demonstrated that the enzyme with a pH optimum of 6.1-6.3 disappears in homogenates of human cancer tissue (lung and mammary gland), and increase in its activity is seen at pH optimum 4. Kakizoe et al., in his study, observed six types of iso-enzymes in the rat liver, numbered Type I to Type VI.[43] Some reports have also shown the altered levels of β-Glu enzyme in the patients suffering with cutaneous cancers, prostate carcinoma and hepatomas as well.[41-43] Further research is wanting to understand the exact pattern of this enzyme.

Creatine Kinase (CK)

Creatine kinase (CK) is an enzyme that conjugates phosphate group to creatine in the presence of adenosine triphosphate (ATP), the hydrolysis of same reaction brings the muscle contraction.[44-46] This enzyme exists in three different forms and each isoform is specific to heart, brain, skeletal muscle, and other tissues.[44,45] The CK enzyme is a dimer composed of subunits (Fig. 1) derived from either muscle (M) or brain (B). Three iso-

enzymes have been identified: striated muscle consists of subunit M and designated as MM and it is predominantly present in the blood, heart tissue consists of subunit M & B and designated as MB, and brain consists of subunit B and designated as BB.[44,47] The CK concentrations in serum are directly proportional to the muscle mass and thus males to have increased levels when compared to females.[45] Individuals affected with muscle dystrophies and also people affected with acute kidney failure, altered levels in serum CK levels are seen.[44]

Figure 1: Characteristic features of creatine kinase iso-enzymes.

Iso-enzyme	Subunit make up of iso-enzyme	Tissue from which iso-enzyme has originated
CK-MM	MM	Striated muscle
CK-BB	BB	Brain
CK-MB	MB	Heart Tissue

Enolase (ENO)

Enolase is a glycolytic enzyme, which catalyzes the conversion of 2-phosphoglycerate to phosphoenolase pyruvate.[48] It was identified first by the researchers Lohmann & Meyerhof in the year 1934.[49] Studies have reported three subunits (Fig. 2) in the ENO enzymes which are α, β, and γ. These three subunits make up five iso-enzymes belonging to ENO family.[50,51] ENO1 is also called as αα or non-neuronal enolase (NNE) present in hepatic tissue, nervous tissue, and fat tissue. ENO2 is also called as γγ or neuron-specific enolase (NSE) present in nervous tissues abundantly. ENO3 is also called as ββ or muscle-specific enolase (MSE) present in muscle tissue in very high levels. Literature showed the increase of ENO concentrations in terms of tumor severity, existence of cancerous growths in the neuronal tissues, and in the individuals suffering with myocardial infarctions.[52-54]

Figure 2: Characteristic features of enolase iso-enzymes.

Iso-enzyme	Subunit make up of iso-enzyme	Tissue from which iso-enzyme has originated
ENO1	Aα	Liver, Kidney, Spleen, Adipose tissue
ENO2	Bβ	Muscle
ENO3	Γγ	Nervous tissue

Glucose-6-phosphatase (GluP)

Glucose-6-phosphatase (GluP), is an enzyme that helps in gluconeogenesis process where it is useful to produce glucose during the fasting state.[55-57] It removes phosphate group from the glucose-6-phosphate, thus producing glucose and present only in liver tissue.[57] Absence or deficiency of this enzyme leads to Glycogen storage diseases (Type 1, Type 1A, and Type 1b).[56] Until now three iso-enzymes have been identified in humans, Glucose-6-phosphatase-α, Glucose-6-phosphatase-β, and Glucose-6-phosphatase-2.[58] It is reported that two iso-enzymes Glucose-6-phosphatase-α and Glucose-6-phosphatase-β have found to shown the similar effect of gluconeogenesis.[59] On the other hand, isoform Glucose-6-phosphatase-2 have found to show no effect on gluconeogenesis but has significant role in the pancreatic insulin secretion.[60] Studies have shown altered levels of this enzyme in the patients affected with glioblastoma and colorectal carcinoma.[61,62]

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) enzyme convert lactate to pyruvate and back, with the help of co-factor NAD^+ to NADH and present in almost all living cells.[63-67]

LDH enzyme structure is made of four subunits and all of them have similar molecular weight (32kD) with a slight variation in amino acid sequence in each chain.[65-67] Each subunit is composed of either H or M polypeptide chain.[63-64] Different combinations of these two subunits or polypeptide chains give rise to five isoforms of LDH enzyme (figure 3). Therefore, the five combinations are H4, H3M, H2M2, M3H and M4 varieties and all these isoforms are seen in all individuals.[63,67] All these iso-enzymes can be isolated with the help of electrophoresis technique maintaining the pH at 8.6.[64] H polypeptide chain denotes heart and M polypeptide chain stands for muscle (Fig. 3)[65], so M4 form is seen in skeletal muscles while H4 form is seen in heart.[67] In normal conditions, iso-enzyme LDH-2, which is composed of combination of polypeptide chains of H3 and M1 is more in blood than iso-enzyme LDH-1 which is having a composition of 4 polypeptide chains of H. However, this normal pattern is altered and called flipped pattern when LDH-1 is higher than the LDH-2 iso-enzyme in patients affected with myocardial infarction.[63-68] Lately a report by van Wilpe et al[69] has reported the importance of LDH enzyme as a marker of diminished anti-tumor activity and another report by Bittar et al[68] in suggested the importance of LDH in cancer therapy. The reports[68,69] demonstrated that the increased LDH levels in the tumor is due to the hypoxia and the levels of LDH in the cancer therapeutically treated patients can be taken as the progression of the disease.

Figure 3: Characteristic features of LDH iso-enzymes.

Iso-enzyme	Subunit make up of iso-enzyme	Tissue from which iso-enzyme has originated
LDH-1	H4	Heart muscle
LDH-2	H3M1	Red blood cell
LDH-3	H2M2	Brain
LDH-4	H1M3	Liver
LDH-5	M4	Skeletal muscle

Prostate Specific Antigen (PSA)

PSA is an enzyme normally produced by the glandular tissue of the prostate and secreted into the seminal fluid.[70-75] PSA was first isolated by Chu in 1980.[70,71] It is a glycoprotein with a molecular weight of 32kD.[71] The main function of the PSA is to liquefy seminal coagulum.[70] Two iso-forms of PSA have been observed, one is complexes with alpha-1-antitrypsin and the other one is free PSA which is not bound.[71] Physiologically, bound PSA is present in the blood predominantly than free PSA.[72,73] Although the isoforms of PSA are not diagnostically available, but research through the years studies have been published. The studies reported three iso-enzyme forms of PSA.[74,75] Lately, research through the years has reported three PSA isoforms, 10%–30% of PSA is unbounded as free PSA (earlier mentioned) in the serum and composed of various isoforms.[74-77] First iso-enzyme is named as ProPSA, which comprises 33% of free PSA and increases with cancer, while the second is termed as BPSA, which is regarded as a nicked form of PSA, is secreted from the transitional zone and makes up 28% of free PSA. The third isoform is termed inactive or intact PSA (iPSA), which decreases with cancer.[74-77]

CONCLUSION

The purpose of this review was to view the latest improvements in the field of iso-enzymes from the past decades and see how the patterns of the iso-enzymes are changing in patho-physiology of different diseases. It is clear from the research reviewed that some enzymes like ALP, CK, and LDH have clear specific patterns in particular in some diseases. In the future studies, pattern of such iso-enzymes should be put into thought process in other diseases rather than in already known diseases. Further in some cases, despite the roles of adenosine deaminase, enolase and β-Glu have been studied for several years, the physiological role is still remains obscure in-terms of their respective iso-enzymes pattern in various diseases. In such cases, it is important to conduct more studies across the globe on the results and derive the facts to a conclusion.

Conflict of Interest

The authors have no conflict of interest among them.

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