Review Article

Enzymes in clinical medicine: An overview

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Enzymes are biocatalysts and because of their remarkable properties, they are extensively used in medical diagnosis. Researches in the last two decades have concentrated more on enzymes such as creatine kinase–MB, alanine transaminase, aspartate transaminase, acid phosphatase, alkaline phosphatase etc. for clinical applications. Enzymes are the preferred markers in various disease states such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc. They provide insight into the disease process by diagnosis, prognosis and assessment of response therapy. Even though the literature on the use of enzymes in various disease conditions has accumulated, a comprehensive analysis is lacking and hence this review.

Keywords: Biomarkers, Biosensors, Diagnostics, Enzymes

Introduction

The global market for medical enzymes has been estimated at \$6 billion in 2010 and it is expected to grow at a compound annual growth rate of 3.9%, to reach \$7.2 billion in 2015 (BCC Research report, June 2011). "Diagnostic enzymes" refers to enzymes used for diagnosis or prognosis. Enzymes, like other proteins, are synthesized by body tissue to meet their metabolic needs. They are not always tissue - specific. More than one tissue/organ may synthesize one or more enzymes. Enzymes are preferred in diagnostics, because of their substrate specificity and their activity can be quantitated in the presence of other proteins. Extensive literature on the diagnostic application of enzymes is available; but, the information is scattered and a comprehensive review is lacking. Hence, this study aims to present a critical review on the application of different enzymes in medical diagnosis.

Why enzymes in medical diagnosis?

Disease states usually lead to moderate or extensive tissue damage depending on the time of onset and severity of the disease. Such conditions are usually associated with the release of enzymes specific to the diseased organ or tissue into circulation and this results in an increase in activity of such enzymes in body fluids[1]. Thus, measurement of enzymatic activity in serum/plasma and other body fluids has been employed in the diagnosis of diseases[2].

The application of enzymes can be broadly classified into (I) enzymes in diagnosis and (II) enzymes in diagnostics–biosensors.

(I) Enzymes in diagnosis (Table 1)
1. Bone diseases, autoimmune and inflammatory disorders

Alkaline phosphatase (ALP)—ALP, functioning under alkaline pH, is classified under hydrolases and it removes phosphate groups from nucleotides and proteins. In children, serum alkaline phosphatase levels are considerably higher than in adults and it correlates with the rate of bone growth. ALP values are slightly higher in men than in women, but after age 60, the enzyme value is equal or higher in women. ALP concentrations are increased during puberty, pregnancy and after menopause[3].

The increase in the level of serum ALP indicates an increased osteoblastic activity or when there is active bone formation as in the case of PAGET's disease or rheumatoid arthritis. Other pathological conditions that may result in high levels of ALP also include rickets, osteomalacia, hyperthyroidism and hyperparathyroidism.

Low activities of ALP are far less common and are more likely related to a genetic condition or nutritional deficiency. Hypophosphatasia is a rare disorder characterized by low levels of serum ALP activity resulting in abnormal phosphorylated metabolites and varying skeletal abnormality[4].

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Cathepsin D—Sohar *et al.*[5] report that elevated level of lysosomal cathepsin D is associated with progression of rheumatoid arthritis. Significant increase in the level of cathepsin D has also been reported in collagen-induced arthritis in rats[6]. Govindaraj *et al.*[7] have observed a significant increase in cathepsin D level in endotoxin induced experimental periodontitis rats.

Gelatinase B—Gelatinase B (also called as Matrix metalloproteinase-9 [MMP-9]) is a secreted enzyme that regulates cell-matrix composition. It belongs to the gelatinase subfamily of the MMPs and therefore, its main substrate is gelatin (a denatured collagen). MMP-9 is produced by selected cell types, including keratinocytes, monocytes, tissue macrophages,

Table 1—Enzymes in diagnosis

S.No.	Disorder/disease state	Enzyme(s)	References
1	Bone diseases, autoimmune and inflammatory disorders		
	Rheumatoid arthritis	Alkaline phosphatase	3
		Cathepsin D	5, 6
		Gelatinase B (MMP-9)	9
		Lysozyme	12
		Tartrate-resistant acid phosphatase	14
	Periprosthetic joint infection	leukocyte esterase	11
2	Cancer		
	Breast cancer	Cathepsin D	22
	Breast cancer	Lactate dehydrogenase	31
	Bone metastasis	Alkaline phosphatase	18
	Bone metastasis	Tartrate-resistant acid phosphatase isoform 5b	13
	Hepatocellular carcinoma	Alanine transaminase	19
	Gastric cancer	Glucose-6-phosphate dehydrogenase	29
	Prostate cancer	Prostatic acid phosphatase (PAP)	16
	Premalignant lesions in colon, thyroid, brain, liver, breast and prostate	Cysteine cathepsins	24
	Germ cell malignancy	Lactate dehydrogenase	21
3	Diabetes (type 2)	Alkaline phosphatase	36
4	Gaucher's disease	Acid phosphatase	37
5	Liver disease		
	Jaundice/hepatitis	Alanine transaminase	42
	Obstructive liver	Alkaline phosphatase	3
	Liver fibrosis	Aspartate transaminase	43
	Liver damage	Lactate dehydrogenase - 5	45
6	Myocardial infarction	α-amylase	52
		Creatine kinase - MB	46, 48, 49
		Gelatinases A and B	51
		Glycogen phosphorylase - BB	45
		Lactate dehydrogenase - 1	45
7	Pancreatitis	Amylase	53
		Lipase	54
8	Dental disorders	Aspartate transaminase	56
		Cathepsin D	7
9	Renal disorders	Urinary lysosomal glycosidases	58
		Urinary lysozyme	60
10	Skin disorders	Lipase	61
11	Schizophrenia	Butyrylcholinesterase	62
12	Intracerebral hemorrhage	Aspartate transaminase	63

polymorphonuclear leukocytes and by a variety of malignant cells. MMP-9 appears to be involved in a variety of pathologic processes that occur in autoimmune diseases[8]. Norga *et al.*[9] have observed that human MMP-9 is a marker enzyme for rheumatoid arthritis. Faber-Elmann *et al.*[10] report that MMP-9 plays a role in the pathogenesis of systemic lupus erythematosus and measurement of plasma/serum activity levels of MMP-9 may provide important information when monitoring patients.

Leukocyte esterase—Parvizi *et al.*[11] have stated that detection of leukocyte esterase in synovial fluid is an extremely valuable addition to the physician's armamentarium for the diagnosis of periprosthetic joint infection. The leukocyte esterase colorimetric strip has the advantages of providing real-time results and being simple and inexpensive, it has the ability to rule out or confirm periprosthetic joint infection.

Lysozyme—Lysozyme hydrolyses glycosidic bonds in the cell wall of peptidoglycans. Tornsteinsdottir *et al.*[12] have shown that serum levels of lysozyme are elevated in rheumatoid arthritis patients, which serve as an indicator of monocyte/macrophage activity.

Tartrate-resistant acid phosphatase (TRAP)—TRAP is a glycosylated monomeric metalloenzyme expressed in mammals, with a molecular weight of approximately 35 kDa. It is differentiated from other mammalian acid phosphatases by its resistance to inhibition by tartrate, molecular weight and characteristic purple colour. TRAP is found in osteoclasts and released into circulation during bone resorption. TRAP level is increased in rheumatoid arthritis, osteoporosis and metabolic bone disorders[13]. Significant rise in the level of TRAP has been noticed in the serum of arthritic rats induced with type II collagen[14].

2. Cancer

Acid phosphatase (ACP)—Five important ACPs viz., lysosomal, prostatic, erythrocytic, macrophage and osteoclastic are found in humans, which differ widely with tissue and chromosomal origin, molecular weight, amino acid homology, sequence length, and resistance to L(+) tartrate and fluoride[15].

ACP level in male prostate gland is 100 times more than in any other body tissue. ACP assay is carried out to check whether prostate cancer has metastasized and also to monitor the prognosis. ACP assay is supplemented by the prostate specific antigen (PSA) test. Kirschenbaum *et al.*[16] have reported that prostatic acid phosphatase (PAP) is strongly expressed by prostate cancer cells, especially in bone metastases.

Alkaline phosphatase (ALP)—ALPs from liver and bone are differently-glycosylated forms of a single gene product. The specific estimation of these forms indicates the organ involved. Abnormal expression of genetically-distinct alkaline phosphatase isoenzymes is valuable in monitoring cancers, particularly germ-cell tumors. These isoenzymes include Regan and Nagao isoenzymes, which correspond respectively to normal placental and placental-like alkaline phosphatases, and the Kasahara isoenzyme which appears to result from the re-expression of a fetal intestinal alkaline phosphatase gene[17]. Delmas[18] has observed that determination of bone specific ALP in serum is a useful parameter for monitoring changes of bone formation, and patients with bone metastases show an increased activity of this isoenzyme.

Alanine transaminase (ALT)—Elevated ALT (also known as serum glutamate pyruvate transaminase [SGPT]) levels are also associated with an increased risk of hepatocellular carcinoma[19].

Cathepsin D—Cathepsin D (CD) is a soluble lysosomal aspartic endopeptidase synthesized in rough endoplasmic reticulum as preprocathepsin D. After removal of signal peptide, the 52 kDa procathepsin D (pCD) is targeted to intracellular vesicular structures (lysosomes, endosomes, phagosomes)[20]. Schwartz[21] reports that cathepsin D in breast tissue may be useful in predicting women with breast cancer who are at risk for early recurrence. A majority of clinical studies focus pCD/CD level as a tumor marker of breast cancer[22]. According to the recommendation of the American Society of Clinical Oncology, data published thus far are insufficient to use cathepsin D as a marker for management of patients with breast cancer[23].

Cysteine cathepsins (CCs)—The best known CCs, cathepsins B, L and H, are distributed ubiquitously and they catalyze protein hydrolysis within the lysosomes. Enhanced expression of CCs has been demonstrated in many human tumors, including breast, ovary, uterine cervix, lung, brain, gastrointestinal, head, neck and melanoma. Upregulation of cathepsin B has been observed also in premalignant lesions in colon, thyroid, brain, liver, breast and prostate[24]. CCs have also been implicated in inflammatory diseases, such as inflammatory myopathies, rheumatoid arthritis, and periodontitis. Hence, CCs play a potential role as diagnostic and prognostic markers in cancer as well as in certain inflammatory disorders[25]. Hersze´nyic *et al.*[26] have stated that cysteine and serine proteases may also

have a role as tumour markers in the early diagnosis of gastrointestinal tract tumours.

Cyclooxygenase-2 (COX-2)—COX-2 is not expressed in normal tissues[27]. Studies have shown that the expression of COX-2 is an early event in tumorigenesis and it plays a role in tumor progression[28]. Therefore, COX-2 is a molecular target for early detection and prevention for a variety of cancers.

Glucose-6-phosphate dehydrogenase (G6PD)—G6PD is an important enzyme for the maintenance of membrane integrity. Wang *et al.*[29] report that overexpression of G6PD is closely related to progression of gastric cancer and might be regarded as an independent predictor of prognosis for gastric cancer. Devi *et al.*[30] have demonstrated that leukocyte G6PD could serve as a diagnostic and prognostic tool in acute non-lymphocytic leukemia (ANLL) and chronic myeloid leukemia (CML). The study reports that G6PD level has been found to be significantly decreased in majority of the patients with ANLL while it is increased in all CML patients.

Lactate dehydrogenase (LDH)—An important challenge in the management of cancer patients is the early identification of the individual who might develop recurrence following 'curative' primary therapy. LDH alone or in combination with tumor markers or other factors may be used for this identification. LDH is perhaps the most common enzyme used for prognosis. It is a validated prognostic marker for germ cell malignancy of testis and it is also a key for monitoring patients during treatment and on surveillance. LDH is a valuable prognostic marker in lymphoma, leukemia and colon cancer. Patients can be stratified into treatment protocols based on LDH activity[21]. Radenkovic[31] further adds that activity of LDH in tumor tissue along with mammographic characteristics could help in defining aggressive breast cancers.

Tartrate-resistant acid phosphatase—Cancer metastasis to bone is considered as a terminal event. Biochemical markers of bone metabolism are potentially useful to diagnose metastatic bone disease and to monitor treatment response in cancer patients. Tartrate-resistant acid phosphatase isoform 5b (TRACP 5b) is a biochemical marker of osteoclast number and activity. Mounting evidence has demonstrated serum TRACP 5b as a useful marker of bone resorption and therefore bears clinical applicability in diagnosis and management of bone diseases. It is a biomarker for diagnosis as well as prognosis in various cancers with high incidence of bone metastasis including breast, prostate, lung, and multiple myeloma[13].

Thymidine kinase—Thymidine kinases (TK) have a key function in the synthesis of DNA. Two isoenzymes have been characterized: TK1 is cell cycle-dependent and present in the cytoplasm whereas TK 2 located in mitochondria is cell cycle-independent. The diagnostic and prognostic role of TK1 has recently been investigated. TK1 levels in serum (STK1) and tumor tissues might be helpful for screening and monitoring of human malignancies[32]. TK1 is converting thymidine to thymidine monophosphate, and is related to DNA replication and cell proliferation. The expression of TK1 in tumor tissues is correlated to pathological stages and clinical grades of carcinomas of esophagus, lung and in premalignancy of breast ductal carcinoma. STK1 level could monitor the out-come of tumor therapy by correlation to remission (breast carcinoma, non-Hodgkin's lymphoma), relapse (breast carcinoma) and survival (non-Hodgkin's lymphoma) of patients. It could also predict the risk of development of neoplasia at early stage[33]. Chen *et al.*[34] report that based on an investigation using 35,365 volunteers in China, those showing elevated STK1 levels are three to five times at higher risk for developing malignancies. Zou *et al.*[35] report that STK1 can be used as a potential marker for monitoring patients after surgery with gastric or other cancers.

3. Diabetes (Type 2)

Alkaline phosphatase (ALP)—Kocabay *et al.*[36] have stated that in addition to conventional risk factors such as age, diabetes and female sex, higher levels of ALP may be considered as a risk factor linked to hepatic fibrosis in patients with non-alcoholic steatohepatitis (NASH) and type 2 diabetes.

4. Gaucher's disease

Acid phosphatase (ACP)—Robinson and Glew[37] have reported that increased ACP activity is observed in the serum and tissues of patients with Gaucher's disease, an inborn error of cerebroside metabolism.

Chitinases—The human immune system is capable of recognizing and degrading chitin, an important cell wall component of pathogenic fungi[38]. There are some known human chitinases that have chitinolytic activity viz., chitotriosidase (CHIT-1), acidic

mammalian chitinase (AMCase), as well as multiple noncatalytically active chitinases called chi-lectins[39]. The functions of CHIT-1 and AMCase are unknown, but they are thought to aid in the defense of chitin containing pathogens. Chitinase assay, which measures the presence of chitinase activity via cleavage of the fluorogenic substrate 4-methylumbelliferyl chitotriosidase, shows that CHIT-1 levels are elevated several hundred-fold in the plasma of patients with Gaucher's disease. Therefore, CHIT-1 is now being used as a biomarker for the diagnosis of Gaucher's disease[40]. AMCase is unique in that it functions effectively in acidic pH environment. Consistently, it has been found highly expressed in the stomach, intestinal tissue, and more recently, it is being studied as a biomarker for asthma and other hypersensitivities[41].

5. Liver diseases

Alanine transaminase (ALT)—Increased serum level of ALT indicates a severe liver disease, usually viral hepatitis and toxic liver necrosis. Kim *et al.*[42] have 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.

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 obstructive jaundice[3].

Aspartate transaminase (AST)—AST, also known as serum glutamate oxaloacetate transaminase (SGOT), is a pyridoxal phosphate (PLP) dependent enzyme. Significant increase in the serum level (10-100 times normal) of AST indicates severe damage to liver (viral hepatitis or toxic liver necrosis) or heart cells (MI). Lesmana *et al.*[43] have reported that AST to platelet ratio index (APRI) could be a much cheaper alternative and a useful marker to screen liver fibrosis in the primary care setting when transient elastography is not available.

Gamma glutamyl transferase (GGT)—GGT catalyzes the transfer of amino acids from one peptide to another amino acid or peptide. This enzyme is sometimes referred to as a "transpeptidase". Specifically, it catalyzes the transfer of a gamma glutamyl group to another acceptor. Hepatobiliary disease is the predominant source of increased serum GGT activity. Increases are associated with all forms of primary and secondary hepatobiliary disorders. Elevations are

moderate (2 to 5 fold) with diffuse hepatic cell injury due to toxic or infectious hepatitis. Cholestasis due to intrahepatic or extrahepatic biliary obstruction causes higher serum levels (5 to 30 times normal level). Increases occur earlier and persist longer than ALP in cholestatic disorders[44].

Lactate dehydrogenase (LDH)—Isoenzymes of LDH aid in diagnosis of many diseases. Mair[45] have reported that liver disease is indicated when there is an elevation in the level of LDH-5.

6. Myocardial infarction

Creatine kinase- MB (CK- MB)—Chest pain can result from indigestion or from a serious heart problem. When the heart muscle dies during myocardial infarction (MI), it releases many molecules into the blood stream, one being creatine kinase (CK). Khan *et al.*[46] have reported that serum CK levels are significantly higher in patients with acute infarction than that of control. CK is shown to exist in three molecular forms *viz*. MM, MB and BB. While total CK is recognized as nonspecific, CK-MB is the most specific, accurate and cost-effective means of detecting MI. Compared with other tests for MI diagnosis, CK-MB has the advantage of early increase, high sensitivity as well as specificity. It invariably shows a rapid increase in serum in the early hours after admission with chest pain. Use of CK-MB as a marker helps to diagnose MI within 6 h of the onset of symptoms and >60% of infarctions are detectable within the first hour of admission in the cardiac intensive care unit[47]. Hemalatha *et al.*[48] have shown that CK-MB level measured by mass assay, is elevated significantly in serum on day I after myocardial infarction in rats, induced by coronary artery ligation. A significant elevation in the level of CK-MB has been observed in the heart effluent during myocardial ischemia and reperfusion in isolated rat hearts[49].

During recent years, CK-MB activity assays have been replaced by CK-MB mass assays which measure the protein concentration of CK-MB, rather than its catalytic activity. Enzyme immunoassays have become the choice for measuring CK-MB in the laboratory because analytical interferences which lead to false positive test results are less frequent[45].

Glycogen phosphorylase BB (GPBB)—Glycogen phosphorylase is a glycolytic enzyme which plays an essential role in the regulation of carbohydrate metabolism by mobilization of glycogen[50]. Among the three isoenzymes viz., glycogen phosphorylase LL

(GPLL) [liver], glycogen phosphorylase MM (GPMM) [muscle] and glycogen phosphorylase BB (GPBB) [Brain], the isoenzyme BB is the predominant isoenzyme in myocardium[45]. A rapid rise in blood levels of GPBB can be seen in MI and unstable angina. GPBB is found to increase between 1 h and 4 h after the onset of chest pain in MI patients.

Lactate dehydrogenase (LDH)—While specific enzymes are diagnostic in some cases, isoenzymes such as LDH are equally important. An increase in total serum LDH activity could result from damage to the heart muscle, skeletal muscle, pancreas or liver. To differentiate the tissue damaged, the levels of individual isozymes of LDH are determined. A significant increase in the serum level of LDH-1 indicates that heart muscle has been damaged as in myocardial infarction[45].

Gelatinases—Gopcevic *et al.*[51], suggest the role of gelatinases A and B as biomarkers of early stage of acute myocardial infarction along with membrane damage parameters.

Alpha-amylase—Shen *et al.*[52] have reported that high initial salivary alpha - amylase activity is an independent predictor of acute MI in patients presenting to the emergency department with chest pain.

7. Pancreatitis

Amylase—Agarwal *et al.*[53] have reported that elevation of total serum amylase is a sensitive marker of acute pancreatitis, within 24 h of onset of symptoms. However, after the first hospital day, it is the least sensitive of the enzymatic tests.

Lipase—Level of lipase in serum can be used as a diagnostic tool for detecting conditions such as acute pancreatitis and pancreatic injury[54]. Acute pancreatitis usually occurs as a result of alcohol abuse or bile duct obstruction. Although serum trypsin level, ultrasonography, computed tomography and endoscopic retrograde cholangio-pancreatography are more accurate laboratory indicators for pancreatitis, serum lipase and amylase levels are still used to confirm the diagnosis of acute pancreatitis[55].

8. Dental disroders

Aspartate transaminase (AST)—Periodontal disease is one of the most common inflammatory diseases of the oral cavity, characterized by the progressive destruction of the alveolar bone and soft tissues surrounding the teeth. Kamma *et al.*[56] report that higher levels of AST are noticed in the gingival crevicular fluids of diseased sites. The relationship between AST levels in saliva and gingival crevicular fluid (GCF) with periodontal disease progression has been studied in a large number of patients. The results show that when compared between the AST levels in saliva and GCF, GCF is shown to have a higher level of AST. Hence, AST level in GCF could serve as a potential biochemical marker for periodontal disease progression[57].

9. Renal disorders

Lysosomal glycosidases—Gatsing *et al.*[58] have investigated three urinary lysosomal glycosidases viz., N-acetyl-β-D-glucosaminidase [NAG] (β-hexosaminidase), β-glucuronidase and β-galactosidase and they are found to be of particular diagnostic value in the early detection of diabetic nephropathy, with NAG being the most useful indicator. Karakani *et al.*[59] also state that measurement of urinary NAG in diabetic patients serves as a biomarker for screening diabetic renal dysfunction.

Lysozyme—Lysozyme hydrolyses glycosidic bonds in the cell wall of peptidoglycans of some microorganisms and thereby aids in host defence. Severini and Aliberti[60] have reported that the concentration of lysozyme in urine is a sensitive indicator of renal damage. Urinary lysozyme concentration is significantly higher in patients with chronic renal failure and for its quantitative determination, a HPLC method has been reported.

10. Skin disorders

Lipase—Higaki and Morahashi[61] have examined *Propionibacterium acnes* lipase in skin diseases. Butyric acid production in axillary seborrheic dermatitis (ASD) is higher than in other dermatitis and that in acne vulgaris (AV) is significantly higher than in controls. *P. acnes* lipase is the pathogenic factor in AV and fatty acids produced by lipase might be the pathogenic factor in ASD.

11. Schizophrenia

Butyrylcholinesterase—Butyrylcholinesterase (BChE) is an enzyme that has been investigated for its putative role in neurodegenerative and neuropsychiatric disorders. Mabrouk *et al.*[62] have shown that patients with schizophrenia have higher plasma BChE activity than controls. In patients with schizophrenia, BChE activity does not differ with age, alcohol status and clinical sub-types, and it is not correlated to duration of illness. The increase in BChE activity could be related to the pathophysiology of schizophrenia.

12. Intracerebral hemorrhage

Aspartate transaminase (AST)—Kim *et al.*[63] have reported that elevated serum AST level may be an independent predictor of intracerebral hemorrhage (ICH).

(II). Enzymes in diagnostics–Biosensors (Table 2)

A biosensor is a device consisting of a biological sensing element connected to a transducer. The transducer can be electronic, optical, electrical, etc. This offers a powerful tool which is radically altering our approach to analytical methods. Enzymes are natural sensors on account of their highly selective nature. Biosensors possess advantages such as reliability, sensitivity, accuracy, ease of handling, and low-cost when compared to conventional assay methods. These characteristics, in combination with the unique properties of an enzyme, render an enzyme based biosensor ideal for biomedical applications.

An enzyme-based electrochemical sensor is formulated by immobilizing a thin layer of enzyme(s) on the surface of the membrane of an electrode. The analyte to be monitored diffuses into the enzyme layer where the catalytic reaction occurs, either consuming a substrate or generating a product that can be detected electrochemically. The electroactive species produced are monitored either potentiometrically or amperometrically, and the electrochemical signal can be correlated to the concentration of the analyte to be measured. The immobilization method may affect the activity of the enzyme, and thus it can contribute significantly to the sensitivity of the biosensor. Immobilization methods that are currently being used include adsorption, cross-linking and self-assembly, whereas materials into which enzymes are incorporated include carbon paste, conducting or nonconducting polymers, and different types of gels[64]. Some widely used biosensors are discussed below.

Glucose biosensor—Diabetes mellitus is a major health problem characterized by increased sugar levels in blood and urine. Patients with diabetes mellitus need to constantly monitor their blood glucose level to detect fluctuations in glucose level that could lead to hyperglycemia and hypoglycemia[65].

Glucose measurements are based on interactions with one of the three enzymes: *viz.* hexokinase, glucose oxidase (GOD) and glucose-1-dehydrogenase (GDH), among which GOD is widely used in biosensors. GOD (β-D-glucose:oxygen 1-oxidoreductase) catalyzes the oxidation of β-D-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide (Fig.1)[66].

Table 2—Enzymes in diagnostics – biosensors

| Compound detected	Enzyme(s)	Disorder/disease state	References
Glucose	Glucose oxidase/glucose dehydrogenase	Diabetes mellitus	72
Lactate	Lactate oxidase	Ischemic myocardium	84
Urea	Urease	Renal disorders	83
Creatinine and creatine	Creatinine amidohydrolase, Creatine amidinohydrolase, sarcosine oxidase	renal, thyroid and muscle function	81
Oxalate	Oxalate oxidase	Kidney stones	85
Glutamate	Glutamate oxidase	Neuropathology	86
Carnitine	Carnitine dehydrogenase and diaphorase	Carnitine deficiency, renal insufficiency, diabetes mellitus etc	87
Theophylline	Theophylline oxidase	Determination of theophylline (eg. In asthma)	88
Cholesterol	Cholesterol oxidase	Atherosclerosis	89
Amino acid (D-serine)	D-Amino acid oxidase	Brain disorders, especially Schizophrenia	90
Acetylcholine and choline	Acetylcholine esterase and Choline oxidase	Neurological problems	91
Bilirubin	Hemoglobin and Glucose oxidase	Jaundice	92
γ-aminobutyric acid	Gabase and Glutamate oxidase	Neurological problems	93
H_2O_2	Horseradish peroxidase	Determination of cerebral peroxides	94
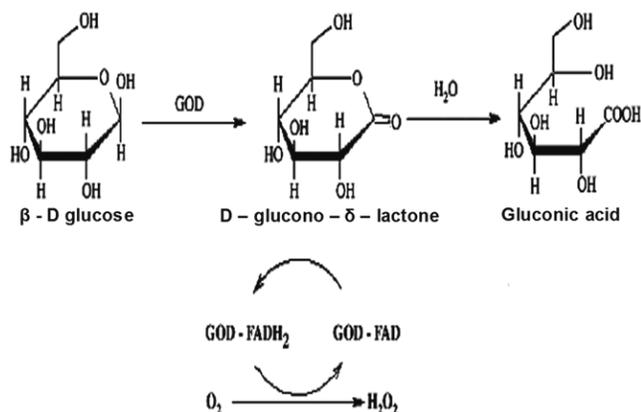
Fig. 1—GOD reaction showing the oxidation of β-D-glucose to gluconic acid

Amperometric biosensors, based on GOD, play a major role in blood sugar estimation. Various GOD based biosensors are reported viz., Disposable strip-type biosensor for blood and serum monitoring[67], Strip type biosensor for blood (GOD-HPR-dye)[68], Miniaturized thermal biosensor for whole blood[69], Glucose sensor for whole blood[70], Glucose biosensor for serum from human blood[71] etc.

Wang *et al.*[72] have employed a glucose micro-biosensor as detector in capillary electrophoresis (CE) for determining the concentration of glucose in human serum. The micro-biosensor is based on the immobilization of the single-walled carbon nanotubes (SWNTs)–glucose oxidase–chitosan biocomposite on a platinized Au electrode by electrodeposition.

Lactate biosensor—Lactate is a key metabolite of the anaerobic glycolytic pathway. Blood lactate concentration is a highly sensitive measure of tissue oxygen deprivation from ischemia, trauma, and hemorrhage. Lactate is formed from pyruvate in muscles and liver and its concentration in blood corresponds to the extent of the oxygen deficit. Lactate level gives an indication of the oxygenation state of tissues, warning of ischemic condition. Lactate sensor is mainly used during surgery and intensive therapy. Lactate sensors may find application in sports medicine and spatial medicine also[73]. Different biosensors for lactate monitoring are based on immobilized lactate monooxygenase (LMO)[74] and lactate oxidase (LOD)[75]. Bienzyme systems such as LOD/LDH[76], cytochrome *b*2/LDH[77], ALT/LDH[78] etc., have also been described.

Development of *in vivo* and *ex vivo* lactate biosensor systems is yet another emerging area. *Ex vivo* biosensors are used with implanted microdialysis or ultrafiltration probes. Lactate from the blood diffuses into the dialysate/filtrate and it is transported outside the body for measurement. *In vivo* biosensors provide a direct measurement of blood lactate concentration, providing rapid response to changes in lactate levels. *In vivo* sensors are placed in the skin or implanted subcutaneously. Response to changes in lactate concentration is rapid, but biocompatibility requirements are more stringent than for *ex vivo* sensors[79].

Creatinine biosensor—Creatinine is the end product of creatine metabolism in mammalian cells. Creatinine estimation is important for diagnosis of renal, thyroid and muscle function. It is also useful for biomedical diagnosis of acute MI as well as for quantitative description of hemodialysis therapy. Creatinine biosensors generally employ creatinine deiminase, which catalyses the following reaction

$$\text{Creatinine} + H_2O \rightarrow \text{N-Methylhydantoin} + NH_4^+$$

Different types of enzymatic biosensors for creatinine detection are available viz., amperometric biosensors, potentiometric biosensor, ion or gas sensitive electrodes etc. Biosensor based on ion sensitive field-effect transistors has been reported by Soldatkin *et al.*[80]. Yadav *et al.*[81] have reported that an amperometric creatinine biosensor based on covalently coimmobilized creatinine amidohydrolase, creatine amidinohydrolase and sarcosine oxidase is shown to successfully determine creatinine concentration in human serum.

Urea biosensors—Renal and liver disorders require a fast and accurate urea measurement in urine or blood samples. Biosensors, based on urease have been used for urea determination. Urease catalyses the conversion of urea to hydrogenocarbonate and ammonium ion.

$$(NH_2)_2CO + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$

Boubriak *et al.*[82] have demonstrated a urease biosensor for the determination of urea in solutions and blood serum by immobilizing urease in a bovine serum albumin membrane on the surface of an ion-sensitive field-effect transistor (ISFET), while de Melo *et al.*[83] have reported the urea biosensor based on immobilization of urease into two oppositely charged clays, which show a greater sensitivity.

Conclusions

Enzymes play a pivotal role in medical diagnostics. They have a wide range of applicability from

analytical detections and immunoassays to biosensors. Enzymes have become the major choice of medical diagnostics because of their high specificity. Though there are a number of research reports on diagnostic uses of enzymes in the last decade, they are yet to reach commercialization. Hence, more research has to be focused on the diagnostic enzymes to reach clinical applications.

Apart from glucose biosensors, the prediction that biosensors would revolutionize clinical analysis has not yet occurred, even though several reported biosensors equal or surpass traditional clinical chemistry methods in important performance metrics such as sensitivity, specificity and time. Also, it could be envisioned that the biosensor technology could be extended to the diagnosis of infectious diseases (both qualitative and quantitative).

Acknowledgement

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