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Biochemical Markers of *In Vivo* Hepatotoxicity

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Abstract

A chemical compound, whether of natural or of synthetic origin, brings about a toxicological effect when its dose is high enough or the duration of exposure is sufficient to cause an alteration in the normal homeostasis of body fluids and tissues. Therefore, the right dose differentiates a toxicant from a remedy. The body detoxifies drugs and other chemical compounds through key organs such as the liver. The liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. These effects can be manifested in the form of hepatic injuries, which take many forms from cellular degeneration and necrosis to cirrhosis or cholestasis to vascular injury. Exposure to hepatotoxicants alters the homeostatic balance of various biological markers that provides a powerful and dynamic approach to understanding the spectrum of liver diseases. These markers offer a means for homogeneous classification of a disease and risk factor, and they can extend one's basic information about the underlying pathogenesis of disease and in drug design.

Key words:

Biological markers; *In vivo* toxicity; ALT; AST; ALP; γ GT; LDH; CK; Bilirubin; α-Amylase; Cholesterol and glucose

Introduction

The liver is the largest organ of the human body that performs multiplicity of vital metabolic functions [1]. Anatomically, the liver is located slightly beneath the diaphragm and anterior to the stomach [1]. It is involved in maintenance of glucose homeostasis, secretion of lipoproteins, and excretion of bile. It synthesizes albumin, prothrombin, fibrinogen and binding protein for iron, copper and vitamin A. Liver parenchyma serves as a storage organ for glycogen, fat and fat soluble vitamins, iron and copper. Catabolically, it is involved in breakdown of various hormones, serum protein, and detoxification of drugs, chemicals and products of bacterial metabolism [2]. The portal vein nourishes the liver with blood containing digested nutrients from the gastrointestinal tract, spleen and pancreas, while the hepatic artery supplies oxygenated blood from the lungs [3]. Approximately 10% to 15% of the total body blood supply will be found in the liver at any one time making it the site most vulnerable to chemical induced toxic injury either through acute or chronic exposures. Ingested metals, drugs and environmental toxicants are the major sources of hepatic injury [3]. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature [4].

Exposure to hepatotoxicants alters the homeostatic balance of various biological markers in body fluids and tissues. Besides, drug induced hepatic injuries have become a major challenge for the pharmaceutical industry and public health, since those injuries are a common cause of drug development termination, drug restrictions, and post-marketing drug withdrawals [4]. Such chemical compounds at some dosages exhibit various toxicological effects or alter the toxicity of concomitantly administered therapeutic drugs [5]. Therefore, clinical evaluation of various biological markers provides a powerful and dynamic approach to understanding the spectrum of diseases. These markers, also, offer a means for homogeneous classification of a disease and risk factor, and they can extend one's basic information about the underlying pathogenesis of disease and in drug design [6].

An ideal marker of xenobioticinduced hepatic toxicity must have a substantial tissue to plasma ratio; is of liver origin (exclusively or predominantly), or its level is affected by a change in liver function (tissue specific); can be reliably measured at sublethal doses of a xenobiotic (highly sensitive); should persist in plasma for several hours to provide a convenient diagnostic time window but not so long that recurrent injury would not be identified (reliability); and it must be easily confirmed to be associated with histopathological or functional changes in the liver (relevancy) [7]. The factors that determine these characteristics and the sensitivity and specificity of each marker are size, cellular localization, solubility, release ratio, clearance, specificity for irreversible injury, and detectability [8].

In this paper, we review various biochemical parameters measured in body fluids that can directly contribute to detecting, quantifying, and understanding the significance of exposure to hepatotoxicants [9,10]. However, the final interpretation of the results of clinical investigations, whether biochemical or of any other category, should be in total context of the disease process and the clinical profile of the patient.

Liver Toxicity

Zimmerman identified four major categories of serum enzymes based on their specificity for and sensitivity to different types of liver injury [11]. The first group contains enzymes such as alkaline phosphatase (AP), 5'-nucleotidase (5'-NT), and gamma glutamyltranspeptidase (GGT) whose elevation in serum appear to reflect cholestatic injury [11]. In contrast, the second group of enzymes includes those that are more sensitive to cytotoxic hepatic injury and are further subdivided into:

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- The enzymes that are somewhat nonspecific since their presence in serum can also reflect injury to extrahepatic tissues and they include aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).
- Enzymes that are found mainly in liver such as alanine aminotransferase (ALT).
- Enzymes that are found only in liver such as ornithine carbamyl transferase (OCT) and sorbitol dehydrogenase (SDH).

The third serum enzyme category contains enzymes that are relatively insensitive to hepatic injury but are elevated with extrahepatic diseases, such as creatinine phosphokinase [11]. The fourth group includes enzymes that demonstrate reduced serum activity in liver disease, such as cholinesterase.

Markers of cholestatic injury

Enzymatic

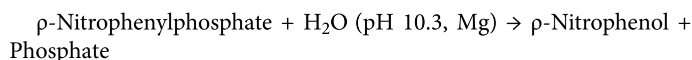
Alkaline phosphatase [AP, ALP] (membrane): Alkaline phosphatase is a membrane-bound enzyme that catalyzes specific chemical reactions within the body especially the hydrolysis of a phosphate group from an organic molecule at an alkaline pH [12]. However, if it is present in serum in large amounts, it is diagnostic of bone or liver disease or a tumor in these organs [13]. Medically, ALP is found in the liver, bone, placenta, and intestine.

A healthy liver continually drain away fluid containing ALP and other substances through the bile duct but for a diseased liver, the bile duct is often blocked accumulating this fluid which eventually escapes into the blood stream. Usually the cells lining the bile ducts in the liver produce the ALP as the first enzyme if the liver disease is primarily of an obstructive nature (Cholestatic) [14]. If the disease on the other hand, is primarily of the liver cells (hepatocytes); the aminotransferases rises prominently. Therefore, these diagnostic enzymes are very useful in distinguishing the type of liver disease, either cholestatic or hepatocellular [15].

Extrahepatic biliary obstruction can be caused if the hepatic or common bile duct is obstructed either partially or completely and the possible causes may include tumor, granulomatous inflammation, abscesses, osteomalacia, benign familial hyperphosphatasemia, pregnancy (3rd trimester), pancreatitis and duodenitis. It is decreased in hypophosphatemia [16]. ALP levels can rise due to pathologic lesions or physiologic causes of the liver. Physiologic cause can be due to normal bone growth in children leading to release of ALP from bone osteoblasts, pregnancy whereby ALP is released from the placenta and intestinal ALP isoenzyme in healthy individuals with blood group B and O [17]. The levels of ALP help in differential diagnosis of hepatobiliary disorders. Patients with extrahepatic cholestasis and primary or secondary liver malignancy are reported to have highest levels and activities of ALP. Intrahepatic cholestasis caused by viral hepatitis, alcoholic hepatitis, drug hepatitis or chemical hepatitis may cause increase in in ALP levels up to three times above the upper limit of the reference range [17]. This occurs during complete extrahepatic obstruction which happens when one has carcinoma of the head of the pancreas or cancer of the extrahepatic bile ducts [17].

Alkaline phosphatase reagent is used to measure alkaline phosphatase activity by a kinetic UV method using a 2-amino-2-methyl-1-propanol (AMP) buffer [18]. In the reaction alkaline phosphatase catalyzes the hydrolysis of the colourless organic phosphate ester substrate, p-nitrophenylphosphate to the yellow coloured product, p-nitrophenol and phosphate. The reaction occurs at an alkaline pH of 10.3. 5 µl of the sample reacted with 250 µl of the reagent. The change in absorbance is monitored at 410 nm and this change is directly proportional to the activity of ALP [18]. The activity is then calculated and expressed in IU/L. The reaction takes place at 37°C for three minutes.

The principal of the reaction is as follows:



ρ-Nitrophenylphosphate + H_2O (pH 10.3, Mg) → ρ-Nitrophenol + Phosphate

(Yellow) (Colourless)

Glutamyl transpeptidase [GGT] (membrane): Gamma glutamyl transferase participates in the transfer of amino acids across the cellular membrane and in glutathione metabolism. It is present in cell cytoplasm and also bound to membranes. It is a carboxypeptidase which cleaves glutamyl groups and transfers them to peptides and other appropriate receptors. It has been shown to be a sensitive marker of cholestasis [19]. It may be used in combination with other tests, to determine the presence and origin of cholestasis. For instance, ALP is increased in hepatobiliary disease and bone disease while GGT is elevated in hepatobiliary disease, but not in bone disease. Most cells show some γGT activity, especially the kidney, liver and pancreas, but most of the serum γGT is derived from the liver.

The physiological function of γGT is unknown, but it could be associated with glutathione metabolism. It mediates intracellular intake of extracellular glutathione which is an important component of antioxidant mechanisms [20]. Glutathione plays an important role in the protection of cells against oxidative stress. Elevation of serum γGT appears to be quite specific for intrahepatic or extrahepatic cholestasis. In liver damage γGT may be used as an indication of chronic change, due to its slower release and metabolism, compared with the transaminases (ALT and AST). As such it is often associated with cirrhosis and marker of alcohol abuse [20].

GGT reagent is used to measure γ-glutamyl transferase activity by an enzymatic kinetic UV rate method. In the reaction, γ-glutamyl transferase catalyzes the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is proportional to the activity of GGT present in the sample and is measured kinetically at 405nm.

The principal of the method is as follows:

L-γ-glutamyl-3-carboxy-4-nitroanilide+glycylglycine Gamma-GT ↔ L-γ-glutamylglycylglycine+5-amino-2-nitrobenzoate

5' Nucleotidase [5'-NT] (membrane): 5′-Nucleotidase (5'-NT) is an intrinsic membrane glycoprotein enzyme in a wide variety of mammalian cells. It catalyzes the hydrolysis of the phosphate group from 5'-nucleotides, resulting in corresponding nucleosides. Is made soluble from membranes by a detergent or bile acids. The reference range of 5'-NT is 2-15 U/L.

Despite its widespread distribution in the human body, serum elevations of 5NT are medically useful in identifying hepatobiliary disease. 5'-Nucleotidase (5NT) levels are elevated the following: obstructive or cholestatic liver disease, hepatitis, intrinsic liver damage, liver malignancy and biliary cirrhosis [21].

Nonpathologic conditions in which 5'-NT levels are elevated include during pregnancy and use of hepatotoxic medications such as

acetaminophen, halothane, isoniazid [INH], methyldopa, and nitrofurantoin. Conditions that cause intrahepatic cholestasis leads to slight increase of 5'-Nucleotidase [21]. Hepatic carcinomas and choledocholithiasis causes up to five times increase of 5'Nucleotidase. Increase in serum levels of 5'-Nucleotidase resembles those of ALP though the elevations persist longer than that of ALP [22]. 5'-Nucleotidase levels do not rise as a result of osteoblatic bone disease and thus its elevations can be used to differentiate osteoblastic bone disease from cholestatic liver disease [22].

The 5'-NT assay is based on the enzymatic hydrolysis of 5'-monophosphate (5'-IMP) to form inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl- N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner.

Non-enzymatic markers

Total serum bile acids: The primary bile acids, cholic and chenodeoxycholic are synthesized in the liver. They then enter the intestinal lumen after meal ingestion where they emulsify fats yielding a greater surface area for pancreatic lipase action [23]. They also promote the aggregation of free fatty acids following fat breakdown forming micelles that maintains a concentration gradient of free fatty acids allowing rapid absorption [23]. They are conjugated with glycine and taurine forming glycocholates and taurocholates before entering the gallbladder for storage.

Secondary bile acids, deoxycholic and lithocholic acids are formed by bacterial deconjugation and chemical alteration of the conjugated bile salts [24]. Both the primary and secondary bile acids are reabsorbed completely from the bowel by passive and active reabsorption into the enterohepatic pathway where they are re-extracted from the blood by the hepatocytes and re-excreted into the bile [24].

The reference interval for total bile acids in serum is 3-30 mg/L (0.8 ~mol/L) (about 0.8 g/dl is excreted in the feces. Total bile acids are increased in acute, chronic, and alcoholic hepatitis, cirrhosis, and are relatively sensitive early marker of cholestasis. Hepatic bile salt uptake and serum bile salt concentrations are sensitive indicators of hepatocellular dysfunction and decreased or altered blood flow in the enterohepatic pathway [25].

Plasma bilirubin (direct and total): Bilirubin and its components play a vital role in evaluating liver function or hemolysis [26]. Bilirubin is mainly formed from the breakdown of heme [27].

Heme → biliverdin → bilirubin → conjugated bilirubin

It is then transported in the plasma loosely bound in albumin to the liver for conjugation. The bound form is not water soluble and is referred to as INDIRECT reacting or UNCONJUGATED bilirubin. The liver then conjugates the indirect bilirubin with glucuronic acid and it is then referred to as DIRECT or CONJUGATED bilirubin which is water soluble and is excreted into the intestine through the biliary system. Some of the direct bilirubin is reabsorbed back into the circulation from the ileum [28].

Acute and severe hemolysis can cause an elevation of indirect bilirubin even in scenarios when the hematocrit and red blood cell counts are often low [29]. Direct reacting hyperbilirubinaemia occurs due to impaired hepatic secretion of bilirubin and/or obstruction to bile flow which can either be intrahepatic, extrahepatic or both. Haemolytic disease may also elevate direct bilirubin since a large quantities of the free bilirubin is conjugated [30]. A slight increase in conjugated bilirubin can occur during hemolytic sickle cell disease due to infarction of the liver. Neonates also have increased serum unconjugated bilirubin a condition known as physiologic jaundice which results from immaturity of the liver hence inability to conjugate bilirubin and increased hemolysis of erythrocytes [31].

Total bilirubin (TBL) is a composite of unconjugated (extrahepatic) and conjugated (hepatic) bilirubin. Increased levels of TBL indicates jaundice and can be due to metabolic problems in the liver associated with reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion [32]. It may also be elevated in acute or chronic hepatitis, cirrhosis, congenital liver enzyme abnormalities (Dubin-Johnson, Rotor's, Gilbert's, and Crigler-Najjar syndromes), fasting and hepatotoxic drugs. Gilbert's syndrome is associated with defects in the transport of unconjugated bilirubin in hepatic membrane or decreased hepatic UDP glucuronyltransferase. Crigler-Najjar syndromes are associated with low levels or absence of UDP glucuronyltransferase thus resulting to increasedvserum unconjugated bilirubin levels [32]. During fasting, metabolic products being utilized for the production of energy preferentially conjugated by UDP glucuronyltransferase in liver cells. Bile duct and pancreas carcinomas cause extrahepatic obstruction of bile duct thus leading to increase in conjugated serum bilirubin. Non-Hodgkin's lymphomas and autoimmune primary biliary cirrhosis may also obstruct bile ducts causing increase in conjugated bilirubin in serum [33].

Markers of hepatocellular injury

Somewhat non-specific enzymes

Aspartate aminotransferase [AST] (cytosol/mitochondria): It is a cytoplasmic and mitochondrial enzyme predominantly found in the liver, heart, skeletal muscles, kidney, pancreas, erythrocytes, lungs and brain tissue [34]. It is a biomarker upon a diseased state or injury to these tissues [35]. AST is responsible for the metabolism (transamination) of aspartate. The amount of AST found in the blood stream corresponds with the number of cells affected by the disease or injury, but the level of elevation depends on the length of time that the blood is tested following the injury [34]. After cell injury, serum AST levels become elevated within eight hours and reach the peak at 24-36 hours, and returns to normal in three to seven days. In cases of chronic (ongoing) cell injury, AST levels remain elevated. Even though the sensitivity of the AST test is believed to be lower than that of ALT, it is still a valuable biomarker of liver disease [36]. AST is used in combination with other enzymes, for example, alanine aminotransferase (ALT); to monitor the cause of various liver disorders such as diagnosing acute alcoholic hepatitis and cirrhosis with an AST/ALT ratio at 2:1. Owing to its more ubiquitous expression in extrahepatic organs, such as the heart and muscle, AST is also diagnostic of other disorders or diseases such as acute pancreatitis, muscle disease, trauma, severe burn and infectious mononucleosis [36].

The aspartate aminotransferase activity is measured by an enzymatic kinetic UV rate method at 340 nm. In the reaction, aspartate aminotransferase catalyses the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate

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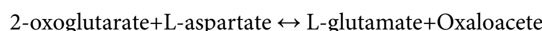
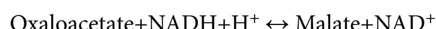
dehydrogenase (MDH) with the concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NAD+). The change in absorbance is directly proportional to the activity of AST. The activity is then calculated and expressed in IU/L.

The principal of the method is as follows:



2-oxoglutarate+L-aspartate ↔ L-glutamate+Oxaloacete



Oxaloacetate+NADH+H^+ ↔ Malate+NAD^+

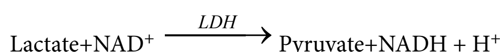
Lactate dehydrogenase [LDH] (cytosol): LDH is an intracellular enzyme which is widely distributed throughout the body and is found at high levels in tissues that utilize glucose for energy; it is therefore not organ specific. As a result, an increase in LDH can reflect damage to a number of different tissues (skeletal or cardiac muscle, kidney, liver) [37]. LDH have five isoenzymes and isoenzyme profiling may help identify specific tissue origin.

Lactate dehydrogenase is a crucial enzyme involved in energy metabolism in muscle, facilitating the production of ATP via glycolysis during oxygen deprivation by recycling NAD^+ [37]. The hypoxic conditions due to effects of extracts stimulated the secretion of LDH as an alternate anaerobic pathway to increase ATP production hence high levels of LDH in blood [37].

LDH levels may be increased whenever there is cell necrosis or when neoplastic proliferation of cells causes an increase LDH production. Erythrocytes have high levels of LDH; therefore, even slight hemolysis can alter the serum activity considerably [38]. Also LDH will diffuse out of the RBCs into the serum, if serum is not separated quickly. Non-haemolysed serum samples must be submitted if valid LDH values are to be obtained. High elevations of LDH are also observed for megablastic anemias, shock, renal infarction, hemolytic conditions, leukemias and liver disease [38]. Although not organ specific, elevated LDH activity indicates tissue damage, and other more specific diagnostic tests may help identify the source. The various isoenzymes of LDH can be identified by electrophoresis and these may also help in identifying the source of tissue damage [39].

The enzymatic kinetic UV test for the quantitative determination of LDH involves a reaction whereby LDH catalyzes the oxidation of lactate to pyruvate coupled with the reduction of NAD^+ to NADH. The change in absorbance due to reduction of NAD is monitored at 340 nm. This change is directly proportional to the concentration of LDH in the sample and is used to calculate and express concentration in IU/L.

The principal of the method is as follows:



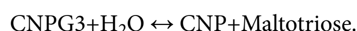
$$\text{Lactate+NAD}^+ \xrightarrow{LDH} \text{Pyruvate+NADH + H}^+$$

Activity of α-Amylase: Alpha amylase is a carbohydrate hydrolyzing enzyme that catalyzes the hydrolysis of large insoluble starch molecules into maltose and finally to glucose, which is the only sugar that can be used by the body [40]. It has an optimum pH of 6.7-7.0. It is secreted by the salivary gland and pancreas, and so present in saliva and serum. Salivary-amylase has been used as a biomarker for stress that does not require a blood draw [41]. Consequently, these enzymes can be important targets in management of postprandial hyperglycemia in type II diabetic patients.

Earlier studies reveal that cigarette smoking causes an increase in both serum and saliva α-amylase activity value. Increased serum α-amylase may also indicate pancreatitis. Individuals with serum amylase level higher than normal could be suffering from renal insufficiency or nephrectomy. Also, high serum amylase activity has been observed in hepatic necrosis and cirrhosis [42] hence the liver is thought to play a role in amylase metabolism. Thus, increase in serum or salivary amylase could be as a result of decreased metabolic clearance of amylase, pancreatitis or parotitis. Parotitis is a salivary disease that is associated with in-crease in S-type isoamylase [40]. Parotitis is usually caused by trauma, stress or surgery to the salivary gland, radiation to the neck area involving the parotid gland and subsequently causing duct obstruction, or calculi of the salivary duct [40].

Amylase reagent is used to measure the concentration of amylase by a kinetic color method using Olympus Autoanalyzer [43]. In the reaction, 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) substrate is reacted with amylase in the serum to release 2-chloro-4-nitrophenol (CNP) from the substrate which is directly proportional to the concentration of amylase in the sample [43]. 3 µl of sample is reacted with 300 µl of reagent and the change in absorbance is monitored at 340 nm, due to reduction of NAD. This change is directly proportional to the concentration of AMY in the sample and is used to calculate and express concentration in IU/L. The reaction takes place at 37°C for three and half minutes.

The principal of the method is as follows:



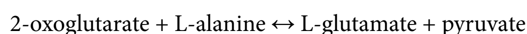
CNPG3+H_2O ↔ CNP+Maltotriose.

Enzymes found mainly in liver

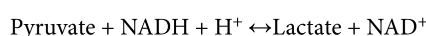
Alanine aminotransferase [ALT] (cytosol): It is a cytoplasmic enzyme predominantly found in the liver and to a lesser extent in skeletal muscles, kidney and heart [34]. It is responsible for transamination or metabolism of alanine. The typical reference range is 10-40 IU/L for males and 7-35 IU/L in females [35]. Upon a hepatocellular injury the enzyme ALT leaks into the extracellular space and enter the blood, wherein it shows a slow clearance rate with a half-life of approximately 42 hrs [35]. A rise in plasma ALT activities is indicative of an injury to the cytoplasmic membrane in the cell. It is a specific biomarker for cytoplasmic and mitochondrial injuries where its high concentration is greater than that of AST [33]. It is increased in acute viral hepatitis (ALT>AST), biliary tract obstruction (cholangitis, choledocholithiasis), alcoholic hepatitis and cirrhosis (AST>ALT), liver abscess, metastatic or primary liver cancer; right heart failure, ischemia or hypoxia, injury to liver ("shock liver"), extensive trauma.

In the assay reaction, the ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamine. The pyruvate then reduces to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinuleotide (NAD). Pyridoxal-5-phosphate is required in this reaction as a cofactor for transaminase activity by binding to the enzyme using Schiff-base linkage [44,45]. The change in absorbance is monitored at 340 nm and this change is directly proportional to the activity of ALT. The activity is calculated and expressed in IU/L. The reaction takes place at 37°C.

The principal of the reaction is as follows:



2-oxoglutarate + L-alanine ↔ L-glutamate + pyruvate



Pyruvate + NADH + H^+ ↔Lactate + NAD^+

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Enzymes almost exclusively located in liver

Ornithine carbamyl transferase [OCT] (mitoch.): Ornithine carbamyl transferase is an enzyme that is localized exclusively in the mitochondrial matrix of the liver parenchymal cells [14] (>97%) and the ileum where it constitute less than 2% [46]. It is involved in urea synthesis where it catalyzes the reaction between carbamoyl phosphate and ornithine to form citrulline and phosphate in the urea cycle as follows:

Ornithine + Carbamoyl-P ↔ citrulline + Pi

The upper reference value in serum is about 20 μ/L. Its activity increases in patients with infectious hepatitis, cirrhosis of the liver, obstructive jaundice, biliary attacks, and cancer of the liver. Its deficiency causes a buildup of ammonia which associated with development of hepatic encephalopathy in which there is decreased mental capacity and eventually stupor, coma, and death [46].

Sorbitol dehydrogenase [SDH] (cytosol): Sorbitol dehydrogenase (SDH) is an enzyme predominantly found in the liver and it's elevation in serum is an accurate indicator of hepatic injury [47]. It is also found in small quantities in the kidney and testes. Marked elevations of SDH are also indicated after acute obstruction of bile flow, hepatitis, cirrhosis and cancer of the liver. It catalyzes the reversible oxidation-reduction involving the interconversion of fructose and sorbitol, a 6-carbon polyhydric alcohol [47].

H^+ + DPNH + Fructose ↔ Sorbitol + DPN^+

Together with aldose reductase-1 it makes up the sorbitol pathway that is believed to play an important role in the development of diabetic complications [48]. The first reaction of the pathway (also called the polyol pathway) is the reduction of glucose to sorbitol by ALDR-1 with NADPH as the cofactor. SDH then oxidizes the sorbitol to fructose using NAD (+) cofactor.

Other markers of hepatocellular injury

Cholesterol: Cholesterol forms an integral part of cell membranes, providing unique physical properties to the membranes to facilitate cellular functions [49]. It is also the precursor of cholesterol ester, bile acids and steroid hormones and its synthesis is primarily dependent on hepatocyte metabolism but may occur in any tissue.

Cholesterol absorption is dependent upon biliary secretion and the hydrolytic activity of pancreatic lipase. Despite lack of fat hydrolysis during pancreatic insufficiency, some cholesterol absorption still occurs due to bile salt emulsification [50].

The amount of cholesterol from dietary sources and hepatic synthesis is under close homeostatic control with the rate of synthesis inversely proportional to absorption. The dietary cholesterol ester is majorly utilized in the liver and is lost in form of bile acids, free cholesterol or its derivatives in bile [51].

Hypercholesterolemia is indicated in inherited lipoprotein deficiencies (beta lipoproteinaemia and alpha lipoprotein deficiency), intestinal malabsorption/maldigestion, and advanced liver disease [52].

Hypercholesterolemia may occur independently or with lipaemia and hypertriglyceridemia. Hypercholesterolaemia with lipaemia may occur with hypothyroidism, hyperadrenocorticism, diabetes mellitus, acute pancreatitis, hepatic disease (especially if extrahepatic biliary obstruction), protein-losing enteropathy and nephritic syndrome (glomerulonephritis) [53]. It may also occur in the postprandial period and with starvation. It is also a feature of steatitis.

In uncontrolled diabetes mellitus, increased serum cholesterol accompanies a general increase in serum lipids [54]. This is due to the absence of insulin which results in decreased mobilisation of serum triglycerides into fat deposits. In diabetes mellitus, hypercholesterolaemia is also due to decreased activity of insulin-dependent lipoprotein lipase and decrease of cholesterol. The resultant hypercholesterolaemia predisposes to atherosclerotic vascular disease [54].

Hypercholesterolaemia usually accompany liver disease but is of limited prognostic value. In obstructive biliary disease, it may be due to retrograde flow through the biliary system and/or bile salt retention [55].

Excessive serum cholesterol levels are also associated with glomerular disease or nephrotic syndrome especially membranous glomerulonephritis and amyloidosis. Its pathogenesis is related to hypoproteinaemia, since serum cholesterol and serum albumin concentrations maintain an inversely proportional relationship [56].

Glucose: Blood glucose provides a major source of fuel for many cells apart from fatty acids and proteins.

Glucose hemeostasis is normally maintained by the breakdown of dietary carbohydrates and a rather complex system of endogenous production system such as glycogenolysis (glycogen broken down to glucose in the liver) and gluconeogenesis (formation of glucose from biochemical precursors) [57]. The maintenance of normal plasma glucose requires delicate balance of glucose availability with glucose utilisation.

Glucose regulation involves many hormones including glucagon, epinephrine, cortisol and insulin. Insulin, a dominant glucoregulatory factor is secreted by B cells of the Islets of Langerhans in the pancreas and is primarily involved in the stimulation of glucose utilisation by a variety of insulin-sensitive tissues including muscle, fat and liver [58].

Glucagon, epinephrine and cortisol are all glucose-raising hormones. Glucagon acts on the liver by stimulating both glycogenolysis and gluconeogenesis. Epinephrine limits both glucose utilisation and stimulates its production. Cortisol antagonises the effects of insulin and limits both the stimulation of glucose utilisation and the suppression of glucose production by insulin [59].

Major causes of hyperglycemia include administration of corticosteroids, diabetes mellitus, pancreatitis drugs such as morphine, administration of fluids containing dextrose or glucose, acromegally and glucagonoma. Hypoglycemia occurs majorly during insulin overdose and over administration of other hypoglycemic agents such as sulfonylureas, tolbutamide; prolonged starvation; hepatic disorders; hyperinsulinism/insulinoma ; extrapancreatic tumours and neonatal hypoglycaemia [59].

Enzymes relatively insensitive to hepatic injury

Creatine phosphokinase [CPK] or creatine kinase (CK)

Creatine kinase is the enzyme responsible for regeneration of ATP [41]. CK transfers a phosphate from creatine phosphate to ADP to form ATP. Creatinine phosphate is the major form of high energy phosphate required by muscle for contraction. Creatine kinase is useful in diagnosing skeletal muscle or cardiac muscle degeneration. The half-

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life of CK is very short and levels decrease rapidly. Increases in CK can be caused by skeletal muscle damage and excessive exercise, muscle anoxia, from prolonged recumbency, myositis, nutritional myopathy, and myocardial infarction [60]. Healthy and active persons show higher values of serum CK activity. Moreover, CK values are lower in women than men and are usually lower in the morning than in the evening. Hospitalized patients tend to have lower values, possibly because bed rest decreases the amount of enzyme released from skeletal muscle [61]. Reference interval for total CK activity lies between 130-253 μ/L in humans [61]. Such wide variation occurs due to age, sex and race and in normal animals, only high increases in CK activity are clinically significant [62].

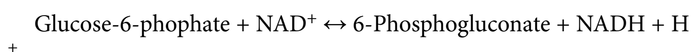
AST is also useful in the diagnosis of muscle damage and can act as a prognostic indicator. Elevated CK values indicate that muscle damage is active or has recently occurred. If the CK continues to remain elevated, the muscle damage is continuing. If elevated AST levels are associated with decreasing or normal CK levels, the muscle damage is no longer active [62].

Creatine kinase activity is determined using the method described by Oliver and Rosalki which is based on the primary reaction that is catalyzed by CK resulting in production of creatine and ATP. The ATP produced in the primary reaction is then used in a coupled enzymatic glucose assay using hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD). The production of NADPH in the indicator reaction is monitored at 340 nm and is related to CK activity within the patient specimen [62].

The principal of the method is as follows:

ADP + Creatinine phosphate ↔ Creatinine + ATP

ATP + Glucose ↔ ADP + Glucose-6-phophate


Glucose-6-phophate + NAD^+ ↔ 6-Phosphogluconate + NADH + H^+

Enzymes that demonstrate reduced serum activity in liver disease

Choline Esterase [ChE]

Acetylcholine esterase and butyrylcholine esterase: Cholinesterases belong to esterases and represent important animal enzymes with multiple biological functions. Acetylcholinesterase (AChE) is an enzyme that terminates acetylcholine neurotransmitter function at the synaptic cleft of cholinergic synapses. It is therefore, needed for the proper functioning of the nervous systems of humans. It predominates in the healthy human brain. It is inhibited by organophosphates and carbamates.

Butyrylcholinesterase (BuChE) is a plasma enzyme that catalyzes the hydrolysis of choline esters, including the muscle-relaxant succinylcholine and mivacurium [63]. Butyrylcholinesterase is produced by the liver and circulates in the blood. It is involved in the breakdown of certain drugs, including muscle relaxant drugs called choline esters that are used during general anesthesia. These drugs are given to relax the muscles used for movement (skeletal muscles), including the muscles involved in breathing, and are often employed in emergencies when a breathing tube must be inserted quickly [64]. It also helps protect the body by breaking down certain toxic substances such as pesticides, solanine and poisons that attack the nerves before they reach the nerves. The enzyme may be involved in the transmission of nerve signals as well.

Both AChE and BuChE therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioral and global functioning characteristic of Alzheimer's disease [65]. The two enzymes differ in substrate specificity, kinetics and activity in different brain regions. Both enzymes have become recently the target for the most frequently used therapy of AD--cholinesterase inhibitors [65].

Cholinesterase inhibitors are drugs that prevent the degradation of acetylcholine (ACh) by acetylcholinesterase [66]. By preventing the inactivation of ACh, cholinesterase inhibitors enhance the actions of ACh released from cholinergic neurons. The cholinesterase inhibitors can be viewed as indirect-acting cholinergic agonists (Table 1).

Parameters	Hepatocellular	Hepatobiliary	Mitochondrial
5'-nucleotidase (5-NT)		X	
Alanine aminotransferase (ALT)	X		
Alkaline phosphatase (ALP)		X	
Aspartate aminotransferase (AST)	X		
Gamma glutamyltransferase (GGT)		X	
Glutamate dehydrogenase (GLDH)	X		X
Lactate			X
Lactate dehydrogenase (LDH)	X		
Ornithine carbamyltransferase (OCT)	X		
Sorbitol dehydrogenase (SDH)	X		
Total bile acids (TBA)		X	
Total bilirubin (TBILI)		X	
Unconjugated bilirubin (UBILI)	X		

Table 1: Clinical chemistry variables that are considered useful in identifying liver toxicity.

Conclusion

Exposure to hepatotoxicants causes homeostasis alterations of various biochemical markers in body fluids or tissues. Clinical laboratory assessment of such biomarkers is important in diagnosis, treatment or prevention of disease, and for greater understanding of the disease process as a result of toxic effects of the chemical compound [67]. Moreover, since biochemical markers have a more

suitable signal-to-noise ratio, their evaluation is important in pharmaceutical industry as they act as a prerequisite of an optimal toolbox in drug development, identification of those patients at high risk, ensuring inclusion of the appropriate patients for clinical studies, identification of patients who will benefit the most from treatment and early intervention of a pathological condition before disease manifestation.

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