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Biomarkers of Hepatic Toxicity: An Overview

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ABSTRACT

Background: Hepatotoxicity is the foremost issue for clinicians and the primary reason for pharmaceutical product recalls. A biomarker is a measurable and quantifiable attribute used to evaluate the efficacy of a treatment or to diagnose a disease. There are various biomarkers which are used for the detection of liver disease and the intent of liver damage.
Objective: This review aims to investigate the current state of hepatotoxicity biomarkers and their utility in clinical settings. Using hepatic biomarkers, the presence of liver injury, its severity, prognosis, causative agent, and type of hepatotoxicity can all be determined.
Methods: Relevant published articles up to 2022 were systematically retrieved from MEDLINE/PubMed, SCOPUS, EMBASE, and WOS databases using keywords such as *drug toxicity, hepatotoxicity biomarkers, biochemical parameters,* and *nonalcoholic fatty liver disease.*
Results: In clinical trials and everyday practice, biomarkers of drug-induced liver injury are essential for spotting the most severe cases of hepatotoxicity. Hence, developing novel biomarker approaches to enhance hepatotoxicity diagnosis will increase specificity and/or identify the person at risk. Importantly, early clinical studies on patients with liver illness have proved that some biomarkers such as aminotransferase, bilirubin, albumin, and bile acids are even therapeutically beneficial.
Conclusions: By assessing the unique signs of liver injury, health care professionals can rapidly and accurately detect liver damage and evaluate its severity. These measures contribute to ensuring prompt and effective medical intervention, hence reducing the risk of long-term liver damage and other major health concerns.

Introduction

The liver is the major organ responsible for the breakdown of carbohydrates, proteins, and fats. It works in tandem with the spleen to rid the body of worn-out RBCs, produce bile for digestion, and produce lipoproteins and plasma proteins like clotting factors.[1] The liver is responsible for an incredible array of vital functions that keep the body running smoothly and in homeostasis. It plays a role in almost every metabolic process that promotes development, immunity, nutrient uptake, energy production, and reproduction.[2] An amazing feat in maintaining homeostasis[3] is the detoxification of drugs and xenobiotics in the liver by drug-metabolizing chemicals. Council of International Organizations for Medical Sciences states that when liver enzymes exceed the upper range of normal, liver damage develops.[4–6] Both pharmaceutical and nonpharmaceutical agents can cause hepatotoxicity. Individual differences, age, gender, alcohol consumption, smoking, con-

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APAP, N-acetyl-para-aminophenol; APPT, activated partial thromboplastin time; AST, aspartate aminotransferase; CAT, catalase; CBZ, carbamazepine; CK18, cytokeratin 18s; FLI, Fatty Liver Index; FLS, The liver fat score; GDH, glutamate dehydrogenase; GGT, gamma-glutamyl transferase; GLDH, gultamate dehydrogenase; GSTM1, glutathione S-transferase in the mu class; GSTT1, glutathione S-transferase theta-1; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, serum hepatitis B virus RNA; HDL, high-density lipoprotein; HIS, hepatic steatosis index; HLA, human leukocyte antigen; HMGB1, high mobility group box 1; IL-6, interleukin 6; K18, keratin 18; LDL, low-density lipoprotein; miRNA, microRNA; MtDNA, mitochondrial DNA; NAC, N-acetyl cysteine; NAFLD, nonalcoholic fatty liver disease; NAT, N-acetyl transferase; POLG, DNA polymerase subunit gamma; PT, prothrombin time; SOD2, superoxide dismutase 2; TG, triglyceride; TNF-α, tumor necrosis factor alpha; TyG, triglyceride-glucose index; VLDL, very low-density lipoprotein.

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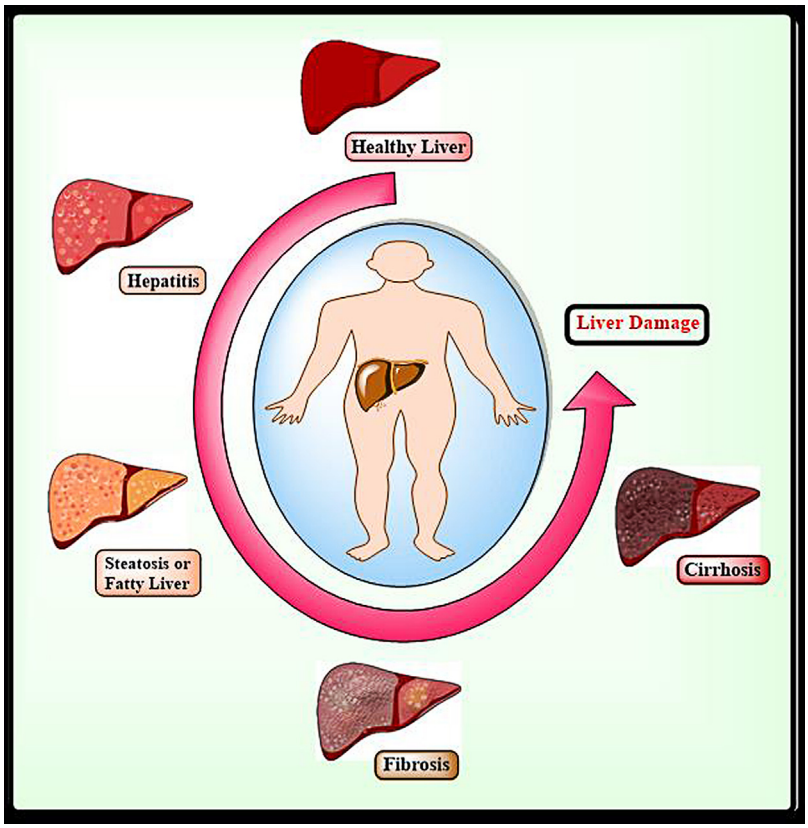

Figure 1. Stages of liver damage in hepatotoxicity.

comitant use of other medicines, previous or underlying liver ailment, and genetic and environmental variables all contribute to an increased risk of liver cancer.[7–9] There are more than 900 drugs known to cause liver damage, making it the leading reason for drug recalls. Five percent of all hospitalizations and half of all acute liver failures are caused by drug-induced liver injury. More than 75% of those with an unusual response to medicine need a liver transplant or pass away.[10] In this article, we will study the current state of hepatotoxicity biomarkers and their utility in clinical settings. Therapeutically, biomarkers may be prioritized in the future.

Epidemiology of hepatotoxicity

Preclinical therapeutic candidate evaluation utilizing animal studies and conventional clinical pathology measures fail to detect up to 40% of potentially hepatotoxic compounds in humans.[11] Sgro et al[12] found in their study 19.1% hepatotoxicity cases per 100,000 in Iceland and 13.9% cases per 100,000 people in France, with 12% hospitalizations and 6% mortality (500 deaths per year in the French general population). An Italian case control study found 4.1% hepatotoxicity cases per 100,000 individuals per year. Hepatotoxicity is reported at 2.3% to 2.4% per 100,000 person in the United Kingdom and Sweden[13,14] and 14% to 19% per 100,000 person in France and Iceland.[12,15] A recent Chinese study found a yearly incidence of 23.8% per 100,000 people in Asia for hepatotoxicity.[16]

Stages of liver damage

Hepatotoxicity is classified according to the severity and intensity of hepatic cell damage and the elevation of hepatic biomarkers. There are various stages of liver damage, which are classified from the initial damage to severe disease as elaborated in Figure 1. Various specific and nonspecific risk factors trigger these stages of the liver.

Types of hepatotoxicity

Hepatotoxicity can be divided into intrinsic reactions (less common) and idiosyncratic reactions (more common). Hepatocellular, cholestatic, or mixed hepatic damage is caused by a 2 to 3 times higher increase in alanine aminotransferase (ALT) or alkaline phosphatase (ALP).[17,18]

Risk factors of hepatotoxicity

Idiosyncrasy, gender, age, alcohol intake, concurrent use of other medicines, smoking, prior or underlying liver illness, and genetic and environmental variables are risk factors.[19,20] Mitochondrial malfunction, decreased cellular respiration, or alterations in fatty acid oxidation have all been linked to hepatotoxicity.[21,22] Damage to hepatocytes can be caused by a variety of circumstances, some of which are presented in Figure 2.

Biomarkers of Liver Disease

There are various biomarkers which are used for the detection of liver disease and the intent of liver damage. Some biomarkers are disease-specific and other are general liver parameters which increase in every liver diseases shown in Figure 3.

Biomarkers in hepatic injury

There are 2 categories of conventional biomarkers for liver injury: first, those that point to a disruption in normal liver function or homeostasis, and second, those that provide unique signs

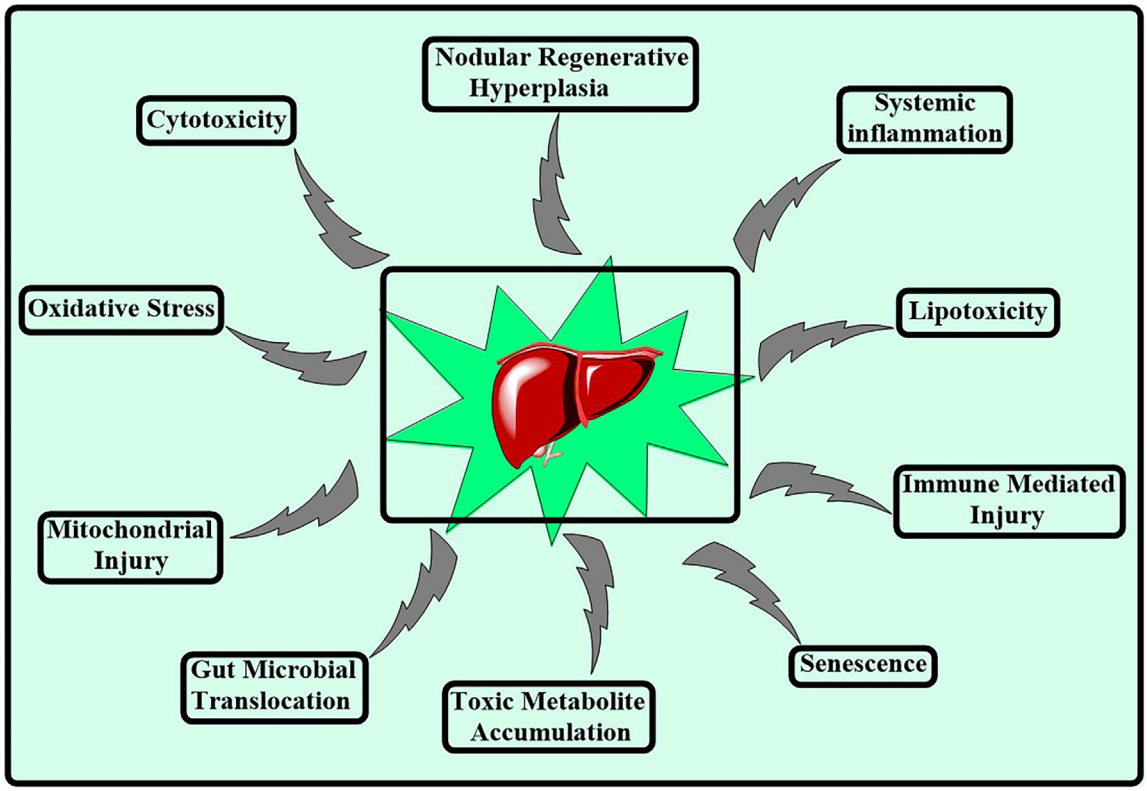

Figure 2. Factors which affect the hepatic cells and cause damage of hepatocytes.

of tissue and cellular damage. The liver is involved in synthesis of proteins, process bile acids and other endogenous chemicals, and excretion of metabolic waste products including bilirubin and urea. Changes in plasma bile acids, plasma total bilirubin, and plasma total plasma protein due to medicines or disorders are conventional indications of impaired liver function. These biomarkers are commonly released into the circulation by wounded or dying cells and hence, can be assessed. Enzymes like glutamate dehydrogenase and gamma-glutamyl transferase as well as ALT and aspartate aminotransferase (AST) fall under this category.[23] Albumin, total protein, triglycerides, and coagulation tests are other accessible options. Although a number of biomarkers are used to detect hepatotoxicity, there remains a necessity for more research in this area.[24,25]

Traditional markers of liver disease

The symptoms of liver illness are often vague and can be mistaken for those of other conditions, making diagnosis a challenging task. Identifying and tracking these conditions requires the use of biomarkers. Liver disease may be detected biochemically by monitoring the levels of a number of enzymes and products of the metabolic pathway that occurs in the liver. Figure 4 describes the categorization of liver disease indicators. These traditional biomarkers[26] elevate according to the type of hepatic disease which is triggered by different risk factors. Various mechanisms of action and homeostasis process involve during the hepatic damage. These liver diseases are categorized according to the form of hepatic damage, necrosis, apoptosis, and generational risk factors.

Various hepatic pathological symptoms of hepatotoxicity are classified based on the elevation of hepatic parameters and the type of hepatic biomarker secretion in the bloodstream. Various stages of liver damage and the degree of hepatocyte damage are given in Figure 5.

Hepatic biomarkers are utilized as noninvasive identifiers for hepatic injury

Aspartate aminotransferase and alanine aminotransferase

ALT and AST are metabolic enzymes and the elevated levels of ALT and AST in the blood are indicative of hepatocyte necrosis and inflammation. The rise of AST is often regarded to be less than that of ALT in viral hepatitis, but both are clinically relevant in detecting acute hepatic damage.[27]

The observation that liver ALT activity is significantly higher than serum ALT activity underlines its primary location within the liver. However, it is also present in smaller amounts in other tissues like the kidney, heart, and skeletal muscles. The difference in the plasma half-lives of ALT (47 hours) and AST (17 hours) is notable, especially considering that ALT is catabolized in the liver.[28]

AST's role in maintaining the NAD^+/NADH ratio and its involvement in synthesizing various essential biomolecules, including purines, pyrimidines, glucose, urea, and proteins, is crucial.[29] The fact that the products of the AST reaction (alpha-ketoglutarate and oxaloacetate) help replenish Krebs cycle intermediates further underlines its metabolic significance. The increase in AST and ALT due to tissue damage, apoptosis, or liver cell injury can be substantial, sometimes up to 50 times the normal levels. Elevated AST levels are associated with a range of conditions including viral hepatitis, alcoholism, cirrhosis, cholestatic syndromes, drug toxicity, myocardial infarction, septic shock, and muscle injuries.[30]

Kunutsor et al[31] found that liver aminotransferases are inversely associated with CVD risk, independent of conventional risk factors, and in an approximately log-linear fashion across the normal and entire baseline aminotransferases spectrum. Adding ALT or AST data to a CVD risk prediction model with known risk variables did not raise the C-index or net re-classification.

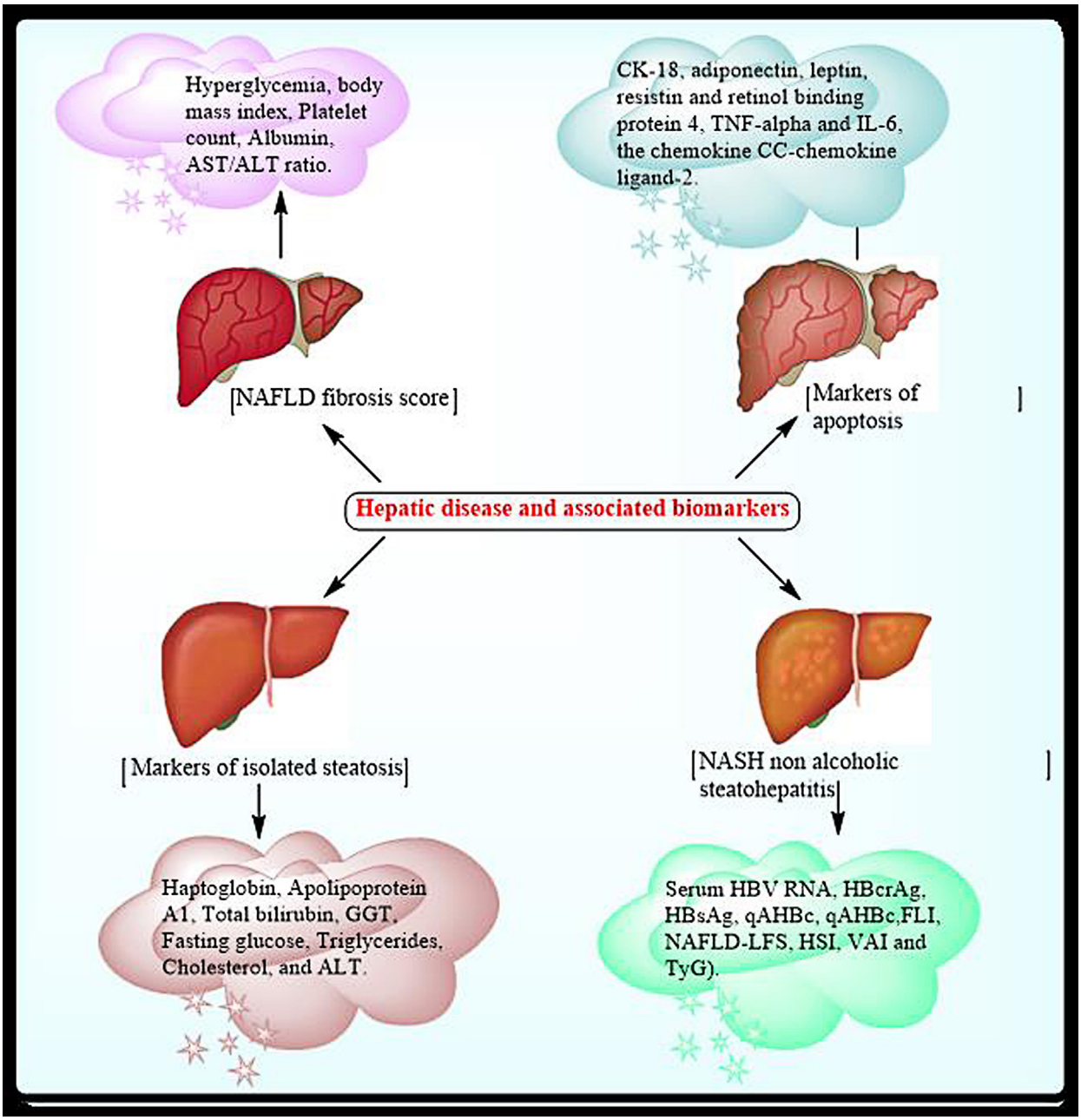

Figure 3. Biomarkers according to disease condition.

Clinical factors associated with serum ALT level

Hepatic-related causes. **Viral hepatitis (mainly hepatitis B virus [HBV] and hepatitis C virus [HCV] infections)**

ALT activity is a sign of liver damage in people with both acute and chronic viral hepatitis.[32] When a person has HBV infection, ALT often goes up during the acute phase of the cytolytic immune reaction and the subsequent ineffective HBV clearance (chronic phase) has shown that ALT activity changes over the course of HBV illness. ALT activity is a crucial measure for figuring out which drugs to be given to HBV patients.[33,34] Thirty-seven percent of HBV-infected people had a lot of scarring and inflammation, but their ALT levels stayed normal.[35]

In HCV, ALT levels are less predictive of disease progression compared to HBV. This is a crucial point in clinical practice, as many patients with chronic HCV infection may have normal or slightly elevated ALT levels, despite ongoing liver damage. This contrasts with HBV, where ALT levels are more closely correlated with hepatic inflammation and damage.[32] The fact that a significant proportion of HCV carriers develop chronic hepatitis leading to permanent liver damage is a major concern. This chronic infection can progress silently, with liver enzyme levels like ALT not always reflecting the extent of hepatocyte damage.[36,37] The study by Ribeiro et al[38] suggests that ALT levels can be indicative of the response to interferon (IFN)-based therapy in HCV. The correlation between a decrease in ALT and a reduction in HCV RNA at week 4 of treatment provides a useful, noninvasive marker for treatment efficacy. The adjustment of the upper limit of normal for ALT to lower values in the US context helps in better identifying individuals with HCV infection. This is particularly relevant given the high prevalence of HCV and the fact that many infected individuals have ALT levels within the normal range or only mildly elevated. The study by Giannini et al[39] points out that hepatic hypoxia (50%) and pancreatobiliary illnesses (24%) are more common causes of hepatitis-like biochemical alterations than viral hepati-

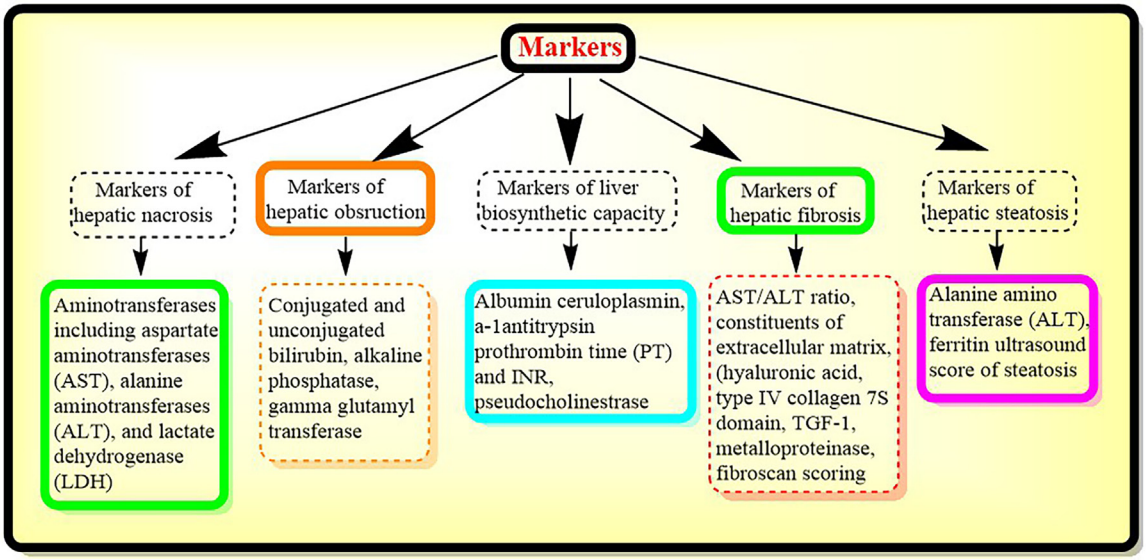

Figure 4. Biochemical indicators of liver disease.

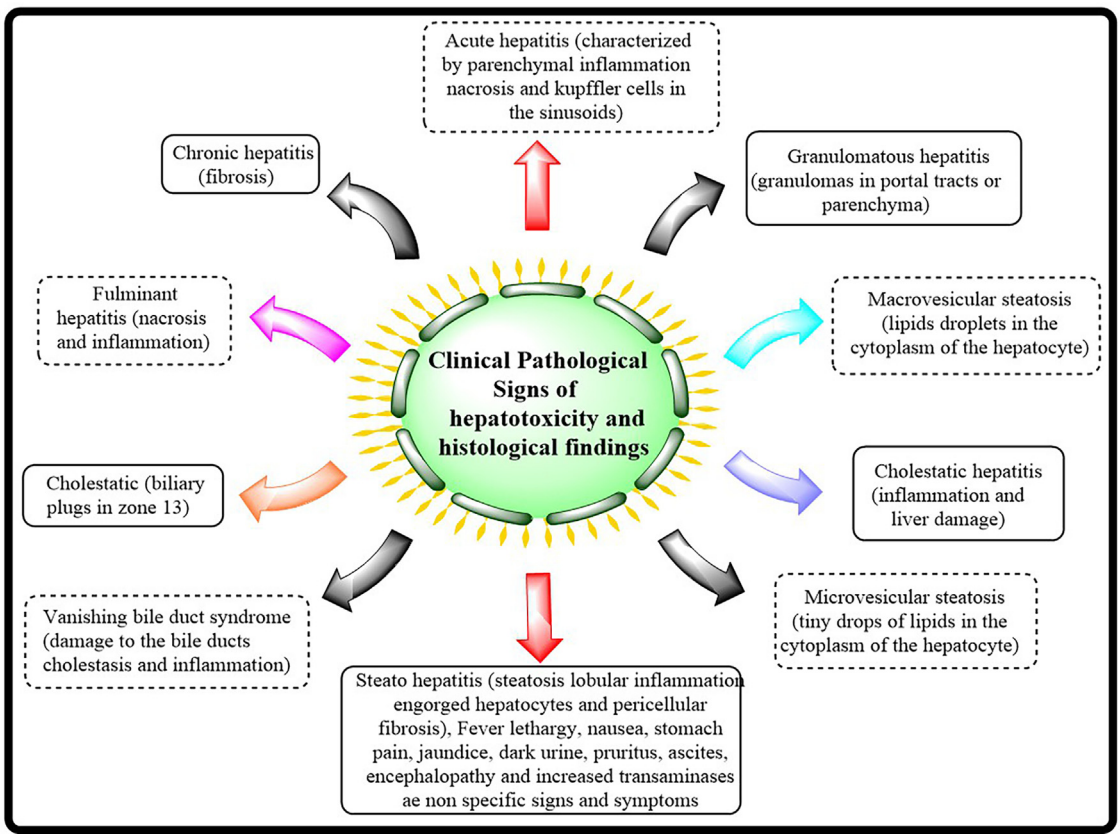

Figure 5. Clinical pathological signs of hepatotoxicity and histological findings.[160,161]

tis (3.6%), or drug-induced liver injury (8.8%). This highlights the need for a comprehensive diagnostic approach in patients presenting with elevated aminotransferases, as the underlying cause can vary widely.[39]

Alcohol intake. Since the liver is the primary site of ethanol metabolism, excessive alcohol consumption results in the most rapid and severe tissue injury.[40] Alcohol consumption may influence ALT activity in a time and dose-dependent manner. Short-term and moderate alcohol consumption did not substantially increase adult ALT levels.[41,42] Moderate alcohol use may affect insulin sensitivity, although it does not significantly elevate ALT levels, especially in normal-weight people.[43,44] Chen et al examined serum gamma-glutamyl transferase (GGT), AST, ALT, mean corpuscular volume (MCV), and carbohydrate-deficient transferrin (CDT) as biochemical markers of chronic alcohol consumption.[45–47] Table 1 displays the sensitivity and specificity of biomarkers for detecting detrimental or excessive alcohol consumption.[48]

There is not a single biomarker that can spot long-term alcohol dependence with enough accuracy. However, combining more than one indicator may make the diagnostic test more accurate.[49] For example, CDT has the best precision for dangerous or excessive

Table 1
Specificity of biomarkers in ALD.

Biomarkers	Sensitivity	Specificity
AST	47%–68%	80%–95%
ALT	32%–50%	87%–92%
MCV	45%–48%	52%–94%
CDT	63%–84%	92%–98%
CDT + GGT	83%–90%	95%–98%
CDT + GGT+MCV	88%	95%

alcohol use, but combining it with GGT and/or MCV makes it much more sensitive.[50]

Hepatotoxic drugs. Diclofenac has been shown to increase ALT levels in the first 4 to 6 months of long-term treatment, but it also has substantial adverse effects.[51] Paracetamol overdose can cause liver enzyme levels to rise to over 20,000 IU/L. The most prevalent laboratory finding for cholestatic hepatotoxicity is an elevated ALP level.[52] A slight increase in ALT has been linked to the use of statins.[53,54] nonsteroidal anti-inflammatory drugs (NSAIDS), antitubercular, antipsychotic, antibiotic, and oral contraceptive drugs cause acute, direct, chronic, idiosyncratic, acute cholestasis, and miscellaneous acute hepatotoxic reactions. Drug-induced hepatic reactions can range from moderate to life-threatening, depending on dosage, treatment duration, and frequency.[44]

Nonalcoholic fatty liver disease. Researchers have found a strong connection between nonalcoholic fatty liver disease (NAFLD) and ALT activity.[55–59] NAFLD is a common, long-lasting liver disease that has been linked to cirrhosis, fibrosis, and liver failure.[60] Reports from different countries show that between 3% and 24% of the general population has NAFLD, and this number is growing along with the number of obese people.[61,62] Most of the time, a moderate rise in ALT that can't be explained is caused by NAFLD.[39,60,63]

Bilirubin

Bilirubin is both an essential heme metabolite and a coordination complex that facilitates iron coordination in numerous proteins. Bilirubin and its breakdown products also give bile, feces, and, to a lesser extent, urine a yellow color.[64,65] Hyperbilirubinemia can be caused by any alteration in the bilirubin metabolism, including excess synthesis, poor liver absorption, conjugation errors, or biliary excretion errors.[66] An extensive study on hepatotoxic patients found that 10% of those with hyperbilirubinemia or jaundice were dead or required a liver transplant.[67,68] Patients with steady coronary artery disease with a low bilirubin level were more likely to have significant adverse cardiac events.[69,70] Fevery J.[66] demonstrated in their study that patient with acute myocardial infarction and a high serum total bilirubin level is more likely to experience a serious cardiac complication or perish from a cardiovascular cause. However, caution is required while interpreting such results due to the fact that cardiac failure after an acute myocardial infarction is common.[66] Ghem et al[71] compared 100 individuals with coronary artery disease to 100 patients with normal coronary arteries and discovered that the control group had considerably higher bilirubin levels. The study also discovered a link between greater levels of ultrasensitive C-reactive protein and an increased risk of coronary heart disease.[71] In diseases like erythroblastosis fetalis, where bilirubin levels are very high because of hemolysis, babies are born with kernicterus and brain problems. Hyperbilirubinemia without conjugation is a sign of liver damage or cholestasis, while an increase in conjugated bilirubin is a sign of cholestasis.[72] All liver diseases reduce the number of hepatocyte cells, which can cause high bilirubin levels.[64] Hyperbilirubinemia can result from an error at any level of bilirubin metabolism, including excess synthesis, reduced liver absorption, conjugation errors, or biliary excretion.[66]

Gamma-glutamyl transferase

GGT is a traditional indicator of liver disease, bile duct issues, and alcohol consumption.[73] Increased GGT, on the other hand, has been linked to a higher chance of stroke, type II diabetes, and coronary heart disease.[74] Dhingra et al[75] showed in their prospective study that higher serum GGT concentrations within the "normal" range were linked to a higher risk of heart failure.[75] The enzyme GGT is involved in the glutamyl cycle and helps make glutathione (GSH) and break it down.[76,77] When GGT levels are elevated, red blood cell membranes become compromised. This results in the release of potentially hazardous transition metals, which can trigger a series of pro-oxidant reactions.[78] Too much peroxidation can lead to oxidative and nitrosative stress, harmful reactive oxygen species or nitric oxide production, and damage to cells, tissues, and DNA.[79] Serum GGT levels are affected by many things, such as alcohol use, body fat, plasma lipid/lipoprotein and glucose levels, and many drugs.[80–82] Extra fat in the liver may make oxidative stress worse, causing GSH to be used up too quickly and GGT output to go up to make up for it. Lastly, a low-grade inflammation of the liver caused by hepatic steatosis[55,83–86] could cause the liver to make more GGT. Fujii et al[87] came to the conclusion that the rate of fatty liver change was higher in the group with abnormal GGT than in the group with normal GGT. Repeatedly high GGT levels raise the risk of fatty liver, and high TG was the only independent predictor in the abnormal-GGT group. Weber et al[88] report on a group of individuals who had hepatotoxicity with a predominant GGT elevation and a rise in liver enzymes below standard criteria.

Alkaline phosphatase

There are 2 types of alkaline phosphatases: tissue-specific and nonspecific. Tissue-specific alkaline phosphatases have been identified in the colon, placenta, and germinal tissue,[89] but tissue-nonspecific ones are essential for therapy identified in the liver, bones, and kidneys.[90] Serum eliminates it after 7 days regardless of liver function or bile duct health. During growth spurts or bone diseases, osteoblast activity increases. Pregnant women may have increased due to placental ALP in the late third trimester.[91] ALP is mostly used to diagnose cholestatic liver disease. Seventy-five percent of people with intrahepatic or extrahepatic cholestasis had a 4-fold or higher upper limit of normal.[92] Serum ALP may *remain* high for a week after biliary obstruction treatment.[93] Wiwanitkit et al[94] found increased serum ALP levels in hospitalized patients with obstructive biliary disorders, infiltrative liver disease, and sepsis. These occurrences also demonstrated the coexistence of cholangitis-carcinoma and local tropical diseases, resulting in an elevation in serum ALP.

Glutamate dehydrogenase

The majority of the time, glutamate dehydrogenase (GLDH) is found in liver lobules, where it is produced in a uniform way.[95,96] Additionally, it is found to a smaller extent in the kidneys, pancreas, brain, and intestines. Muscle tissue had only a small amount.[97,98] GLDH is one of the most important enzymes in the matrix of the mitochondria. Matrix-rich mitochondria are common in the liver, but not in muscle tissue, which has a lot of cristae-rich mitochondria. Studies have shown that GLDH activity is low outside of the liver.[99] ALT is higher in people with muscle problems, but GLDH is not.[95,100] Because of this, GLDH may be a good way to find liver damage in people who already have problems with their muscles.[95] Also, since GLDH has a shorter half-life in human blood than ALT, this biomarker may give a more true picture of the damage to the liver at the same time.[95,97] The half-life of GLDH in blood is between 16 and 18 hours.[95,97]

Arginase

In the liver of ureotelic animals, *arginase* (*L-arginine amidinohydrolase*) catalyzes the hydrolysis of arginine to urea and ornithine. *Arginase* can be separated into 2 types: liver type (*arginase 1*) and extrahepatic type (*arginase 2*).[101,102] The kidney and other extrahepatic organs contain less extrahepatic *arginase* mRNA than the liver, producing most *arginase* mRNA.[103,104] In a study, *Arginase* in rat serum was evaluated in conjunction with serum AST and ALT activity following acute and chronic liver histopathologic injury induced by thioacetamide. *Arginase* I demonstrated the earliest and most significant rise in blood levels among the analyzed enzymes.[105] *Arginase* I was evaluated as a more specific indicator of liver function than standard blood indicators for this model. Serum *arginase* activity peaked on day one after liver transplantation and declined more rapidly than other assays, with a strong and statistically significant correlation to serum AST and ALT activity.[106]

Alpha-glutathione S-transferase

Alpha-glutathione S-transferase (α-GST) is an enzyme that helps get rid of harmful substances from cells. Because it is found all over the liver, has a lot of cytosolic content, and has a short half-life in plasma, it is a better indicator of damage to liver cells than normal biochemical liver function tests.[107] Immunohistochemical studies have shown that α-GST is only found in cells in the liver. Its activity in the blood is said to be a better indicator of liver damage than aminotransferases.[108] The foremost functions of α-GST in the liver are to bind steroids, bile acid, and bilirubin, prevent lipid peroxidation, produce prostaglandins and leukotrienes, and make chemical bonds with electrophiles.[109] Alcoholism, HBV, and HCV viruses can boost the immune system, make free radicals, and turn on detoxification systems. All of these things may cause hepatocytes to produce more α-GST. Because α-GST has a smaller molecular weight and a shorter half-life, it can be used as a more sensitive biomarker of liver function than AST and ALT, which are studied more often.[110] Up to 80% of all α-GST in the body can be found in the liver. In a single hepatocyte, 3% to 5% of all soluble cytoplasmic proteins come from α-GST, but only 0.6% come from ALT. Due to its low molecular weight (52 kDa) and high concentration in the liver, α-GST is quickly released from hepatocytes that have been damaged.[110–113] Also, 5 days after α-GST is released into the plasma, the amount of it returns to normal.

Abdel-Moneim and Sliem[114] found in their work that the mean value of α-GST in HCV patients was much better than that of the control group in terms of sensitivity, specificity, positive predictive value (98%), and negative predictive value (63%). An adjuvant is the α-GST test, which is used to measure the damage to liver cells in HCV patients. But in individuals with normal aminotransferases, it plays a much larger role in the early diagnosis of liver cell injury.[114] Czuczejko et al[109] discovered a positive correlation between α-GST and ALT and AST. This indicates that the measurement of α-GST in conjunction with other markers could be used to corroborate hepatocellular damage. But it would be much more useful if it could detect liver impairment in individuals with normal ALT levels at an earlier stage. It is a much more important part of the early diagnosis of liver cell damage.[114] Czuczejko et al[109] found that there was a positive link between α-GST and ALT and AST indicates that assessing α-GST in combination with conventional markers could be considered as a confirmatory test for hepatocellular damage. But it would be much more useful if it could find liver damage early in people with normal ALT. When you compare the high cost and complexity of the α-GST assay to the low cost and speed of the spectrophotometric methods for ALT and AST, the results do not support using plasma α-GST as a better indicator of liver damage than ALT and AST. Giffen et al[115] concluded that α-GST in the wistar rat is a good sign of this type of induced hepatotoxicity. But compared to the panel of markers already set up in this lab,[115] measuring α-GST provided less information regarding the duration of onset/recovery or the severity of each type of hepatic injury. Abdel-Moneim and Sliem[114] found that the average value of α-GST in HCV patients was much higher than in the control group, with a sensitivity of 82%, a specificity of 85%, a positive predictive value of 98%, and a negative predictive value of 63%. The α-GST assay is used to measure the damage to liver cells in HCV patients. But its role is much more important in people with normal aminotransferases because it helps find early liver cell damage.[116]

Serum F protein translates as a human biomarker of liver injury

The role of a cytoplasmic F protein of 44 kDa has yet to be determined.[117] About 1 ng/mL[118] can be detected via a serum radioimmunoassay. The liver contains the highest concentration of F proteins, while the kidneys contain approximately 14% of the liver's levels. Other body parts have substantially lower concentrations.[119] One possible sensitive and specific liver damage measure is F protein, which has a narrow tissue distribution and a steep concentration gradient between hepatocytes (10 mol/L) and serum (2.5×10 mol/L). Liver histology can be influenced by serum F protein levels as well.[117] The coding sequence for the HCV capsid protein can produce p16 of 16 kDa.[120,121] Frameshifted protein (F) or alternate reading frame protein[122,123] is the name given to this protein. The F protein was found to be cytoplasmic and perinuclear by indirect immunofluorescence.[124] In vitro analysis of peripheral blood mononuclear cells (PBMC) from community health centre (CHC) patients with and without hepatocellular carcinoma (HCC) reveals that the HCVF protein modulates Th1/Th2 cytokine secretions; however, the F protein produces distinct profiles than the core protein. In patients with chronic hepatitis C, the F protein may lead to a Th1/Th2 bias and the subsequent development of HCC to investigate the potential role of HCV F protein-induced Th1/Th2 cytokine patterns in the etiology of HCC in patients with chronic HCV infection. The molecular process and essential phases need further study. This finding has the potential to shed light on the origins of hepatitis C, leading to the development of new preventative and therapeutic anti-HCV medications, and hence inspiring the development of entirely new antiviral therapeutic approaches.[125] Clinical characteristics and frequency of F protein antibiotic use in HCV patients were investigated by Gao et al.[125] Anticore antibodies were present in 95% of patients, anti-F99 synthetic peptide antibodies were present in 36%, and anti-F recombinant protein antibodies were present in 68%. Blood tests were negative for all 40 HBV-infected individuals and all 40 control subjects. Specific antibodies were assessed against synthetic peptides of core, and F99 in different HCV genotypes.[125]

Albumin

The liver can synthesize enough protein to maintain albumin concentrations until 50% parenchymal damage. Plasma albumin measurements assist in assessing severity and longevity. Acute renal disease lowers plasma albumin levels, limiting its utility for this purpose.[126] At this early stage, albumin's metal ion and fatty acid binding capabilities changes, according to Ge et al.[127] They may become early indications of liver malfunction, which could improve liver disease diagnosis and therapy. Antiviral medication can improve liver function and minimize cirrhosis decompensation, which may alter albumin binding function. In a randomized trial by China et al.,[128] albumin infusions to elevate albumin levels to 30 g/L or higher for hospitalized UK patients with decompensated cirrhosis were no more beneficial than the conventional treatment. Tian et al[129] found that severe acute liver inflammation exacerbates glucose metabolism disorders in individuals with hepatitis B-related liver cirrhosis, and high ALB levels are associated

with glucose metabolism disorder regression after acute liver inflammation resolution.

Prothrombin time

Serial prothrombin time (PT) measurements separate cholestasis from severe hepatocellular diseases. Severe hepatocellular injury prolongs PT. Vitamin K malabsorption lowers cholestasis PT.[126,130] Prolonged PT, activated partial thromboplastin time (APTT), and decreased factor V activity increase thrombotic risk but not bleeding risk. All liver illnesses affect the PT.[131] Cirrhosis bleeders have 90% to 100% elevated PT, while nonbleeders have 50% to 55%.[132] Seventy-five percent of viral hepatitis patients had increased PT. PT rises in 80% of obstructive jaundice patients. Cirrhosis affects APTT significantly. Bleeders have 80% elevated APTT and nonbleeders 15%. APTT increased 22.5% in viral hepatitis. Fifty-five percent of obstructive jaundice cases increase APTT. Hypofibrinogenemia observed 55% of cirrhosis bleeders have moderate-to-severe hypofibrinogenemia. Twenty-five percent nonbleeders have mild hypofibrinogenemia. In viral hepatitis and obstructive jaundice, 2.5% have mild hypofibrinogenemia, suggesting fibrinogen has little value.[133] Prajapati et al[134] found that coagulation profile can measure hepatic cell activity and detect cellular harm. In advanced liver cirrhosis, liver parenchyma damage lowers coagulation protein production and increases bleeding risk. Forty-five percent of viral hepatitis patients and 38.5% of alcoholic liver disease patients had elevated PT.

Lipids

Lipids are an essential form of fat used to store energy. They consist of phospholipids, tri-, di-, and monoglycerides, as well as sterols and cholesterol.[135] The precursor high-density lipoproteins (HDLs) and very low-density lipoproteins are produced and released into the bloodstream by the liver, whereas mature particles including low-density lipoproteins, HDLs, chylomicron remnants, and HDL are absorbed by the liver in a receptor-dependent manner. Because the liver is essential for the production and metabolism of cholesterol, patients with hepatotoxicity frequently have abnormal cholesterol levels.[136] Lipoprotein production is decreased in individuals with extensive hepatotoxicity and hence, plasma cholesterol and TG levels decrease noticeably.[137–139] As the severity of hepatotoxicity due to impaired lipoprotein biosynthesis worsens in patients, plasma cholesterol and TG levels decrease significantly, falling from 166.5 to 121.2 mg/dL for cholesterol and from 122 to 92 mg/dL for TG.[136,140] Many studies have shown that HDL can act as an independent predictor of transplant-free death in patients with hepatotoxicity. In hepatotoxic patients, there is also evidence of a strong correlation between HDL levels and liver function. Monitoring lipid profiles, including HDL, can provide valuable information about the overall health status of the liver.

Platelets

Typically, hepatotoxicity is associated with alterations in the hemostatic system.[141] These alterations include decreased plasma concentrations of hepatocyte-produced proteins associated with coagulation and fibrinolysis.[142] Furthermore, thrombocytopenia and platelet dysfunction are common.[143] Platelet production may be diminished due to decreased thrombopoietin production, which appears to significantly contribute to thrombocytopenia in patients with hepatic toxicity.[144]

Serum bile acids

In humans and animals, intrinsic hepatotoxicity alters serum and plasma bile acids. Some bile acids are elevated and associated with ALT. According to a study, nonsurvivors of hepatotoxic patients have higher blood glycodeoxycholic acid levels. Unfortunately, circulating bile acid levels have not been tested in any other clinical scenario, including hepatotoxicity.[145,146]

Other drug-specific biomarkers in hepatotoxicity

Several additional novel biomarkers, such as those listed in Table 3, can be used to assess hepatotoxicity. Fragments of nuclear DNA and mitochondrial DNA (mtDNA) have been studied as potential mechanisms of hepatotoxicity and predictors of patient outcome. Antihistone immune assays can be used to quantify nuclear DNA fragments, while quantitative polymerase chain reaction can be used to quantify mtDNA fragments. Overdosing on N-acetyl-para-aminophenol (APAP) causes an increase in ALT, GLDH, and mtDNA in the blood of mice and humans alike, with mtDNA perhaps being specific for mitochondrial damage.[147]

MiRNAs are one of the most promising hepatotoxicity indicators to date. Multiple organizations have investigated the use of circulating microRNA as hepatotoxicity indicators. Several studies have demonstrated that specific miRNAs, notably miR-122 and miR-192, are elevated in the blood sample of mice and humans following an APAP overdose prior to ALT.[148,149] HMGB1 is a nuclear protein that is involved in gene transcription, nucleosome assembly, and DNA replication and repair.[150] Acetylated HMGB1 is a biomarker of inflammation, whereas total HMGB1 is a sign of necrosis with passive release. K18 is a structural protein that is found in the cytoskeleton. Caspases cleave K18 during apoptosis, revealing a new epitope recognized by an antibody termed M30.[151] Total and caspase-cleaved K18 are elevated in the blood of APAP overdose patients,[69] though total is considerably higher in both APAP overdose and other hepatotoxicity[71] indicating that oncotic necrosis is the predominant cause of cell death.[152]

Many proteins, including argininosuccinate synthetase,[153] paraoxonase 1, glutathione-S-transferase (GST), liver-type fatty acid binding protein 1, cadherin 5,[154,155] macrophage colony stimulating factor receptor, aldolase B,[156] and many more, are regulated by cyclic adenosine monophosphate (cAMP). These additional indicators have not been explored extensively for use in hepatotoxicity at this time. Some of these biomarkers have the potential to shed light on the underlying mechanisms of hepatotoxicity in the future. For instance, macrophage colony stimulating factor receptor has been proposed as an inflammatory biomarker. The molecular significance of these markers in the context of hepatotoxicity, however, has not yet been adequately explored. Additional genetic and nongenetic clinical biomarkers[157,158] for specific drug hepatotoxicity are listed in Table 3.

Discussion

Drugs frequently cause liver damage, but diagnosis and prognosis can be challenging, especially when idiosyncratic reactions are involved. The recently proposed biomarkers and methods for the early diagnosis of hepatotoxicity are promising, but there is variability in the validity, specificity, and sensitivity. A list of clinical biomarkers of liver toxicity is elaborated in given Table 2.

An examination of the current status biomarkers suggests that, in addition to the standard indicators and the enzymatic markers, may provide information to the evaluation of the liver. Aminotransferases rise rapidly as compared to any other hepatic biomarkers and subsequent rapid decline once treatment is stopped demonstrate their sensitivity to detect hepatotoxicity, in contrast to traditional biomarkers, which remain high. So, study suggests that there is a weak association between liver cell damage and plasma amino transferases. Researchers suggest in their research work that in the case of HBV infection, ALT increases often during the acute phase of the cytolytic immune response and the subsequent ineffective HBV clearance (chronic phase).[33] Nonetheless, there is disagreement also reported where 37% of HBV-infected patients had significant fibrosis and inflammation, with persistently normal ALT levels.[35] In contrast to HBV infections, the ALT level is less important for HCV diagnosis and prognosis. More HCV-infected individuals

Table 2
Clinical biomarkers of liver toxicity.[59,126,117,161,162]

Biomarker	*Cellular localization	**Biological activity	***Tissue localization	$Injury	$$Specific damage markers	#Comments	##Disadvantage
ALT	Mitochondria in periportal and cytoplasm	Amino acid reductive transfer from amino acid	Primarily localized to liver	Increased in the presence of liver necrosis, cardiac dysfunction, and muscular damage.	Hepatocellular Necrosis	Standard method for evaluating liver cell damage	• Both enzymes activities can potentially exceed 100 times the upper reference limit. Maximum activity does not correlate with outcome
AST	Cytoplasm and mitochondria periportal	Amino acid reductive transfer from amino acid	Localized in heart, brain, skeletal muscle and liver	Elevated due to liver or extracellular tissue injury	Hepatocellular Necrosis	Less specific than ALT	• Peak enzymes activities do not affect prognosis.
ALP	Cytoplasm	Amino acid reductive transfer from amino acid Formation of new bone	Broad tissue localization	Marker of hepatobiliary injury	Cholestasis	Conventional biliary injury; associated with drug-induced cholestasis in humans	• Elevation tends to be more notable in extrahepatic obstruction than in intrahepatic obstruction • Increase may also be seen in drug therapy
Bilirubin	Cytoplasm and mitochondria	Hemoglobin degradation	Taken up, conjugated in liver, and secreted into bile	Marker of hepatobiliary injury	Marker of hepatobiliary injury and liver function; also increased due to hemolysis	Conventional biliary injury; in conjunction with ALT, better indicator of disease severity in humans	• Bilirubin peaks after marker enzymes • Unable to detect early pathophysiology
GGT	Cell membrane	Gamma-glutamyl transfer cholesterol metabolism	Kidney>liver, pancreas, bile duct	Marker of hepatobiliary injury	Cholestasis, biliary	Conventional biliary injury; high sensitivity in humans, elevation can be caused by alcohol or heart disease	• Usefulness is limited due to lack of specificity • Increased activity of the enzyme is also found in serum of subjects receiving anticonvulsant drugs • example: Phenytoin and Phenobarbital
GLDH	Mitochondrial matrix	Amino acid oxidation and urea production	Liver specific>kidney	Liver damage	Necrosis	More stable enzyme (with storage)	• Low activity outside the liver
Arginase I	Cytoplasm	Arginine metabolism	Liver	Inflammatory process, ROS associated with disease states	Necrosis	Earliest and most easiest rise in blood levels	• Extrahepatic arginase 2 is less in amount
α-GST	Cytoplasm, centrolobular cells	Phase II detox enzyme	Liver specific	Liver damage	Necrosis, prodromal	Better indicator of liver damage	• Triggered by various non-specific substances
Albumin	Endoplasmic reticulum, Golgi apparatus, secretory vacuoles	Protein binding with others	Main constituent of serum total protein	Decreased in blood with chronic liver disease	Liver function	Liver fails to synthesize enough protein, especially albumin	• Even though liver specific, concentrations will be decreased in acute and chronic renal failure.
Prothrombin Time (PT) International Normalized Ratio (INR)	Cytoplasm and mitochondria	Coagulation pathways	Liver	Increased with severe liver injury	Liver function	Liver fails to produce coagulation factors, increased clotting time; international normalized ratio equivalent to prothrombin time	• Cholestasis will decrease PT • Decrease in PT may be secondary to malabsorption of vitamin K Direct biomarkers of CLD • Still in research level and needs validation • Do not have greater significance than routine biomarkers Serum Cytokines • Do not have much diagnostic value. Not organ specific
Lipid	Endoplasmic reticulum	Cell homeostasis	Liver	Hepatitis, NAFLD, and others	Necrosis	Decreased blood lipids in liver failure	Decrease in HDL
Platelet	Cytoplasm	Immune-competent surface markers	Bone marrow	Decreased in blood with chronic liver disease	Infection and inflammation	The liver produces too little protein, especially thrombopoietin.	Low platelet count increases infection risk.
Bile acids	Cytosol, endoplasmic reticulum	Stimulate biliary lipid secretion	Liver, gall bladder	Cholestasis, liver diseases	Infection and inflammation	It raises serum ALP.	Watery stools, fecal incontinence

*Clinical biomarkers are discussed with their cellular localization, **biological activity, ***tissue localization, $injury, $$specific damage markers, #comments and ##disadvantages.

Table 3
Various novel clinical biomarkers for specific drug hepatotoxicity.[157]

Genetic biomarkers	Nongenetic biomarkers
For AILI	
miR-122	GDH
miR-192	mtDNA
11-miRNA panel including has-miR-122-5p	Nuclear DNA fragmentation
miR-382-5p	GLDH
HMGB1	K18
Full length K18	Circular acylcarnitines
	APAP-protein adducts
For antiepileptic drugs	
POLG	Lipid
GSTMI	Ceramides
GSTTI	Sphingomyelins lipid mediators
SOD2 val16Ala	Branched chain amino acid metabolism LPCs
CAT C-262T	CBZ plasma
For antimicrobial drugs	
NAT2*5, *6, *7	T cell profile
HLA-B*57:01	
For anti-TB drugs	
NAT2 *6A	Th17 and T cell expressing IL-10
NAT2*5B, *6A	Isoniazid-specific CD41 T-cell

develop chronic hepatitis with persistent hepatocyte damage. More than 6 out of 10 typical HCV carriers have ALT levels that are normal or very slightly elevated.[36,37] NAFLD is the most typical cause of an unexplained moderate ALT increase.[55–57] The most common laboratory finding in cholestatic drug-induced hepatotoxicity is an increase in ALP. It also rises in osteoblast activity. Cholestasis and severe hepatocellular disorders can be distinguished using serial PT measures. PT will decrease in cholestasis due to vitamin K malabsorption.[126,127] Several variables exacerbate oxidative stress, resulting in GSH overconsumption and a compensatory increase in GGT production. Finally, increased GGT production might be the result of a low-grade hepatic inflammation caused by hepatic steatosis.[55,83,85] *Arginase* I was tested as a more specific diagnostic of liver function than standard blood indicators. Serum *arginase* I attain peak concentration on day 1 after liver transplantation and declined faster than other tests, with a strong and significant association with serum AST and ALT activity.[106] F protein's limited tissue distribution would imply that F protein might be a sensitive and specific marker of liver injury.[117] Plasma albumin is helpful in determining the severity and duration of the condition. However, due to the fact that acute renal illness also causes a drop in plasma albumin concentration, its usefulness for this purpose is constrained.[126] Prolonged PT, APTT, and decreased factor V activity increase thrombotic risk but not bleeding risk but in advanced liver cirrhosis it indicates liver parenchyma damage, which decreases coagulation protein production and increases bleeding risk.[132] As the severity of hepatotoxicity due to impairment in lipoprotein biosynthesis worsens, plasma cholesterol and TG levels (i.e., from 166.5 to 121.2 mg/dL for cholesterol and from 122 to 92 mg/dL for TG levels) show a significant decline.[7] Thrombocytopenia and platelet dysfunction are frequent in hepatotoxic individuals due to reduced thrombopoietin production. In hepatotoxicity, several bile acids are increased and correlate with ALT. Many other drug-specific hepatotoxic biomarkers, both genetic and nongenetic, are also considered in severe drug toxicity. Table 3 lists many additional indicators for drug toxicity based on their genetic and nongenetic origin. However, at the moment, the mechanistic significance of these drug-specific biomarkers has not been well tested. These all biomarker will help us to evaluate signals as indicators of potential liver damage. These indicators will eventually function as bridge markers to track hepatic illness and hepatotoxicity. Noninvasive hepatotoxicity evaluation has been extensively studied and may reduce drug toxicity biopsies. In the recent decade, pathogenetic mechanisms and high-throughput technology have spurred metabolomics research toward noninvasive drug toxicity screening using metabolites.

Conclusion

When it comes to diagnosing and monitoring hepatotoxicity, biomarkers are crucial tools. The hepatic biomarkers ALT, AST, GLDH, GGT, ALP, albumin total protein, lipids, platelets, bile acids, triglyceride, coagulation test, and CTP score were developed to evaluate liver disease severity along with drug-specific biomarkers elevated in particular drug toxicity as mentioned in Table 3. Apart from these drug-specific biomarkers, the mechanistic significance of genetic and nongenetic biomarkers has not been well tested. Despite multiple potential biomarker possibilities from recent research, a generally accepted metabolomics marker for hepatotoxicity or its severity has yet to be established. To establish their therapeutic efficacy and cost-effectiveness, as well as to address aspects like patient variability and the underlying mechanism of liver disease, more research is needed. Early identification and better management of liver injury could improve patient outcomes if accurate biomarkers could be developed and incorporated into clinical practice. The severity of liver damage can be assessed rapidly and reliably by evaluating the specific indication of liver injury.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Simran Thakur: Writing – original draft. **Vishal Kumar:** Data curation. **Rina Das:** Formal analysis. **Vishal Sharma:** Data curation. **Dinesh Kumar Mehta:** Data curation, Supervision.

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