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Comprehensive Review On Biomarkers In Hepatotoxicity: From Conventional Indicators To Omics-Driven Discoveries

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The liver plays crucial role in metabolic homeostasis, detoxification, and biosynthesis, yet it is highly susceptible to damage from xenobiotics, alcohol, metabolic disorders, and infections. Hepatotoxicity, a major contributor to liver disease, progresses from reversible steatosis to irreversible cirrhosis, driven by genetic, environmental, and metabolic factors. Early detection and monitoring of liver injury rely heavily on biomarkers, with conventional options such as aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, bilirubin, and gamma-glutamyl transferase being widely used but limited in specificity. Recent advances in omics technologies have uncovered novel biomarkers, including microRNAs, high-mobility group box 1 (HMGB1), keratin-18, and glutamate dehydrogenase that provide enhanced sensitivity and earlier detection. This review consolidates current knowledge on conventional, emerging, and omics-based biomarkers, exploring their mechanistic roles and clinical potential. Broader adoption of validated biomarker panels could enhance diagnostic accuracy, guide treatment strategies, and ultimately enhance results for liver disease patients.

Keywords: ALT; AST; Biomarkers; Clinical diagnostics; Hepatotoxicity; Hepatic injury; HMGB1; microRNAs; Omics.

The liver is the principal organ essential for metabolizing proteins, carbohydrates, and lipids. Working alongside the spleen, it facilitates elimination of senescent red blood cells, produces bile for digestion, and synthesizes plasma proteins and lipoproteins, including clotting factors.[1] It performs numerous critical functions that maintain the body's equilibrium and overall well-being. Proper liver function is indispensable for nearly all essential metabolic processes, including growth, immune response, nutrient metabolism, energy production, and reproduction.[2] Most hepatotoxic substances damage liver cells by inducing lipid peroxidation, oxidative stress, and elevating serum biomarkers such as alkaline phosphatase, transaminases, bilirubin, triglycerides, and cholesterol.[3,4]

Following are the key functions performed by liver

Regulating nutrient absorption and metabolism from the intestines. 2) Modulating endocrine functions to facilitate

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growth and development. 3) Maintaining energy metabolism (e.g., glycogen storage and gluconeogenesis. 4) Synthesizing and biotransforming proteins, carbohydrates, and lipids. 5) Regulating fluid and electrolyte balance. 6) Producing bile for digestion and eliminating hydrophobic compounds. 7) Supporting immune function via Kupffer cells and acute-phase proteins. 8) Detoxifying drugs and xenobiotics through enzymatic metabolism.

The liver synthesizes approximately 15% of the body's total proteins, with most being secreted directly into the bloodstream. This process begins when transcription factors activate promoter sequences in the DNA, initiating gene expression. Following translation and post-translational modifications, the newly synthesized proteins are released from the sinusoidal surface of hepatocytes into the circulation. Hepatic protein production is tightly regulated by nutritional status and hormonal signals. Among the liver's diverse protein products, are albumin (maintains oncotic pressure and transports molecules) ceruloplasmin (copper transport and oxidation) coagulation factors (fibrinogen, prothrombin, etc.) and fibrinolytic proteins, complement system proteins (immune defense), protease inhibitors (e.g., á1-antitrypsin). Notably, C-reactive protein (CRP), a key acute-phase reactant, is the most commonly measured hepatic protein in clinical practice. While the liver does not store proteins, it efficiently recycles amino acids to sustain ongoing protein synthesis.[5]

Global burden

Alcohol-associated Liver Disease

Global annual per capita alcohol consumption (2016) reached 6.4 liters, with 5.1% of the worldwide population affected by alcohol use disorder. Alcohol remains the leading cause of cirrhosis globally, accounting for nearly 60% of cases in North America, Europe, and Latin America. In recent years, the incidence of alcohol-associated hepatitis has risen significantly particularly among young adults and women. Given the synergistic interaction between alcohol consumption and metabolic risk factors, Europe and North America face a heightened risk of increasing liver disease burden in coming years.

Non-alcoholic Fatty Liver Disease

It impacts approximately 32.4% of people worldwide. Its contribution to global mortality has increased from 0.9% to 0.16% of all deaths. Currently NAFLD ranks as the second most common cause of liver transplants overall and the leading cause in women. Emerging metabolic risk factors in children and adolescents represent one of the most significant impending threats to global health.[6]

Various stages of hepatotoxicity

Hepatotoxicity progresses through distinct clinical stages, with severity and manifestations varying based on etiology including alcohol abuse, metabolic dysfunction, or drug-induced injury, and outlined in Table 1 and Figure 1.

Risk factors of hepatotoxicity

Multiple variables influence hepatotoxicity risk, idiosyncratic factors, age, sex, lifestyle exposures like alcohol intake, smoking, pre-existing liver illness, concurrent use of other medicines, and genetic and environmental influences [Figure 2].[8,9] Mitochondrial dysfunction, impairing energy metabolism, or aberrant lipid metabolism through beta-oxidation have all been linked to the mechanisms of hepatotoxicity.[10,11]

Biomarkers in Hepatotoxicity

Definition and Classification

The NIH defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention".[12] Biochemical markers can be classed based on their purpose (Table/†2).

Biomarkers serve as valuable diagnostic tools in clinical hepatology, complementing other methods to accurately detect liver conditions such as DILI (drug-induced liver injury) and HILI (herbal medicine–induced liver injury) are commonly studied using biomarkers. These indicators usually involve the detection of RNA, DNA, or protein molecules in biological samples, including blood, plasma, and urine.[13-20] The development and implementation of new biomarkers require rigorous validation through testing in confirmed patient populations and comparison with existing diagnostic standards.[16] This ensures their diagnostic accuracy and reproducibility, which clearly differentiate between healthy and sick states and deliver consistent results across laboratories and populations.[14,20]

When properly validated, these biomarkers become essential for early detection of hepatotoxicity, monitoring disease progression, guiding treatment decisions, and ultimately improving patient safety in cases of suspected DILI or HILI.

Conventional biomarkers

Conventional biomarkers for evaluating liver injury can be classified into two primary categories: (a) markers of impaired liver function or homeostasis, and (b) markers of cellular damage. The liver maintains critical physiological functions including protein synthesis, bile acid metabolism, and waste excretion examples include bilirubin and urea. Changes may also be observed in circulating bile acids, overall bilirubin levels, and blood proteins - frequently observed following hepatotoxic drug exposure or in liver disease - serve as well-established indicators of compromised hepatic function.

Indicators of liver cell damage are usually enzymes that leak into the bloodstream when hepatocytes are injured or undergo necrosis. Common examples are alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and glutamate dehydrogenase (GLDH).[21] Additional biochemical parameters such as albumin levels, total protein, triglycerides, and coagulation markers (particularly PT/INR) provide valuable assessment of hepatic synthetic capacity, especially in chronic liver disease.[22, 23]

Standard biochemical markers (Table 3) outline the routine laboratory parameters evaluated during preclinical assessment of drug-related liver toxicity.

ALT and AST

ALT and AST are sensitive biomarkers of hepatocellular injury, with ALT being more liver-specific due to its predominant hepatic localization, while AST is widely distributed across tissues.[24] Their differing plasma half-lives (ALT~47 h; AST~17 h) enhance diagnostic interpretation.[25] Although elevations occur in hepatitis, alcohol-related liver disease, cirrhosis, and drug-induced injury, their limitation is poor specificity, as increases are also seen in muscle damage and myocardial infarction.[26, 27]

The study by Kunutsor *et al*. further noted that despite associations with cardiovascular outcomes, aminotransferases add little predictive value to CVD risk models, emphasizing the need to interpret them alongside other biomarkers and clinical findings.[28]

Gamma-glutamyl transferase

GGT is a validated marker of liver disease, biliary disorders, and chronic alcohol consumption.[29] Elevated levels also correlate with increased risk of stroke, type 2 diabetes, coronary heart disease, and heart failure, even within the normal reference range.[30,31] Biochemically, GGT regulates glutathione (GSH) turnover through the glutamyl cycle, influencing cellular redox balance.[32,33] Its strength lies in its high sensitivity to hepatobiliary injury, oxidative stress, and alcohol intake; however, its major limitation is poor specificity, since elevations are also observed in obesity, metabolic syndrome, cardiovascular disease, and with certain medications. [34-38] In hepatic steatosis, increased GGT reflects oxidative stress–induced GSH depletion, compensatory enzyme upregulation, and inflammation, with fatty liver progression more common in patients with abnormal GGT.[39-44] Persistent GGT elevation further predicts fatty liver risk, and in some hepatotoxicity cases, GGT may rise disproportionately compared with other enzymes.[45]

Alkaline Phosphatase

ALP exists in tissue-specific forms (placenta, germinal tissue, colon) and non–tissue-specific forms present in liver, bone, and kidney. [46] Physiologically, levels may rise during bone growth or pregnancy due to increased osteoblast or placental activity.[47,48]

Clinically, ALP is a useful and sensitive marker for cholestatic liver disorders, with about 75% of patients with intrahepatic or extrahepatic cholestasis showing enzyme levels fourfold above the upper limit of normal.[49] A strength of ALP is its ability to detect biliary obstruction and cholestasis, even persisting for up to a week after resolution of obstruction.[50] However, its limitation lies in poor specificity, since elevations also occur in bone disease, pregnancy, infiltrative liver conditions, and sepsis, making it necessary to interpret ALP alongside other biomarkers and clinical findings. [51]

Glutamate dehydrogenase

GLDH is a mitochondrial matrix enzyme occurring predominantly in liver lobules, with smaller amounts in kidney, brain, intestine, and

pancreas, and minimal presence in muscle.[52-55] Its abundance in the liver's matrix-rich mitochondria, coupled with low activity outside the liver, makes GLDH a highly specific marker of hepatocellular injury.[56] Unlike ALT, which can be elevated in muscle disorders, GLDH remains unaffected, making it particularly valuable for detecting liver damage in patients with concomitant

Table 1. Clinical stages of hepatotoxicity and their common etiological factors

Stage	Description	Causes/Associations
Normal liver	Healthy liver with intact structure; normal metabolism, detoxification, and synthesis.	-
Fatty liver (Steatosis)	Reversible fat accumulation within hepatocytes.	Poor diet, obesity, alcohol, certain drugs.
NAFLD	Fatty liver related to obesity, insulin resistance, and metabolic syndrome.	Sedentary lifestyle, high-calorie intake.
SMAFLD	Fatty liver due to both metabolic issues and alcohol use.	Combined metabolic syndrome + alcohol consumption.
AFLD	Fatty liver caused by prolonged excessive alcohol intake.	Chronic alcohol abuse.
Steatohepatitis	Fat buildup with inflammation and liver cell injury.	Progression of fatty liver disease.
NASH / MASH	Steatohepatitis associated with non-alcoholic or metabolic dysfunction	NAFLD progression with inflammation.
ASH	Alcoholic steatohepatitis.	Chronic heavy drinking.
SMASH	Steatohepatitis from metabolic dysfunction plus alcohol intake.	Metabolic syndrome + alcohol abuse.
Cirrhosis	Irreversible fibrosis with regenerative nodules; severe functional loss; risk of liver failure or cancer.	Chronic liver injury from any cause.

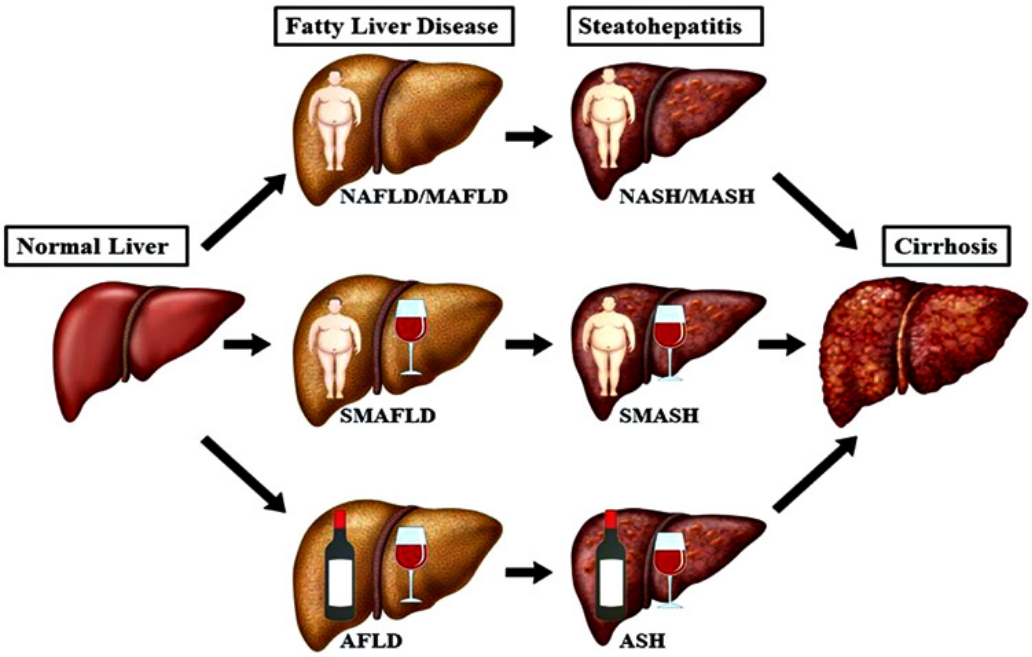

Fig. 1. The sequential phases of hepatic damage in liver toxicity[7]

muscle disease.[52,57] Its shorter plasma half-life (16–18 h) compared to ALT [52,54] also allows more accurate reflection of current hepatic injury. However, GLDH testing is not widely available in routine clinical practice, can be influenced by mitochondrial disorders, and is less validated in large patient populations, which limits its broader clinical application.

Alternative indicators of drug-related liver toxicity

Nuclear DNA and mitochondrial DNA (mtDNA) fragments have been investigated as both mechanistic markers and predictors of hepatotoxicity. Nuclear DNA fragments can be quantified by antihistone immune assays, while mtDNA is measured using quantitative PCR. In N acetyl para aminophenol (APAP) overdose, ALT, GLDH, and mtDNA levels increase in both mice and humans, with mtDNA potentially specific for mitochondrial injury.[58]

Several biomarkers are utilized to detect liver disease and assess the extent of hepatic injury. Some are disease-specific, while others represent general liver parameters that tend to rise across most liver disorders, as shown in [Figure 3].

Table 2. Biomarkers based on their purpose

Biomarker type	Definition	Purpose
Type/ 0–Prognostic biomarkers	Indicators of illness risk or natural progression	Predict the likelihood of disease onset or monitor its natural course
Type/1–Response biomarkers	Characterize biological activity in response to treatment interventions	Assess physiological or molecular changes induced by therapy
Type/2–Surrogate efficacy biomarkers	Determine clinical outcomes and treatment efficacy	Serve as substitutes for direct clinical endpoints in evaluating treatment success
Pharmacodynamic biomarkers	Biological response in a patient after exposure to a medical product	Demonstrate target engagement, confirm drug activity, and guide dose optimization

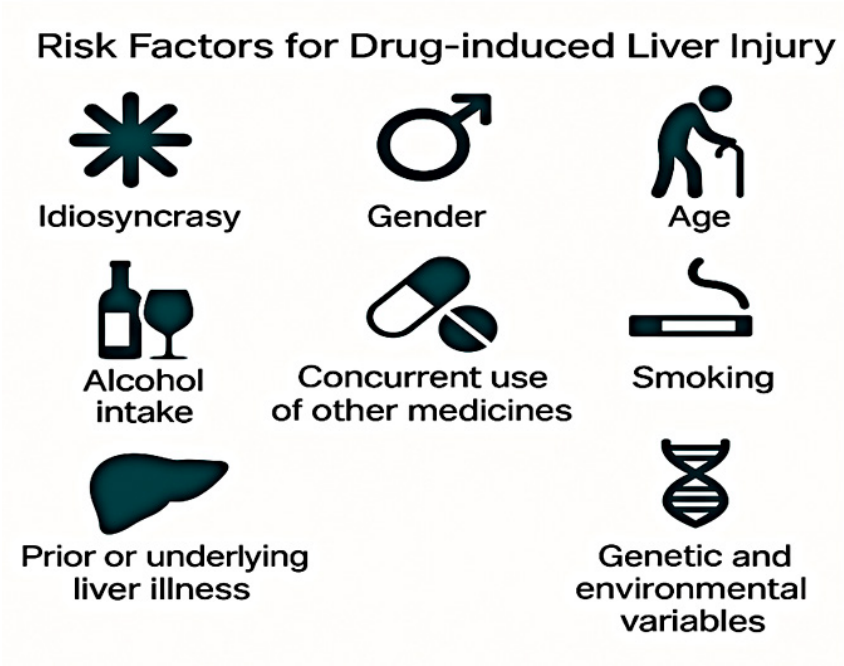

Fig. 2. Common predisposing factors for liver toxicity

Novel biomarkers

MicroRNAs, especially miR-122 and miR-192, are considered highly promising biomarkers, as their levels increase in the blood of both mice and humans following APAP overdose, often preceding the rise of ALT.[59,60] Similarly, HMGB1, a nuclear protein involved in transcription regulation, nucleosome organization, and DNA repair,[61] acts as a marker of necrosis when measured in total form, and of inflammation when present in its acetylated form. Keratin 18 (K18), a cytoskeletal protein, is cleaved by caspases during apoptosis to form a fragment recognized by the M30 antibody.[62] Both total and cleaved K18 are elevated in APAP

Table 3. Principal conventional biomarkers used in the assessment of liver injury

Biomarker	Organ distribution	Diagnostic indication	Associated injury / Role
AST	Found in liver, brain, heart and skeletal muscle	Released into circulation upon hepatocellular damage	Necrosis of hepatocytes
TB (Total bilirubin)	Synthesized and conjugated in liver, excreted via bile	Indicator of hepatobiliary dysfunction; also elevated in hemolysis	Hepatic insufficiency, cholestasis, biliary injury.
ALP	Found in diverse tissues	Sign of hepatobiliary impairment	Biliary stasis
Clotting time	-	Increased with severe liver injury	Liver function
GGT	Liver, pancreas and kidney	Leads to cholestasis and biliary injury	Cholestasis with biliary damage
Bile salts	Secreted via bile ducts	Rises with liver injury and dysfunction	Altered function
Protein levels	-	Declines with advanced liver damage	Reflects liver function
ALT	Hepatocytes	Hepatocellular necrosis; may also rise in cardiac or muscle injury	Cellular death

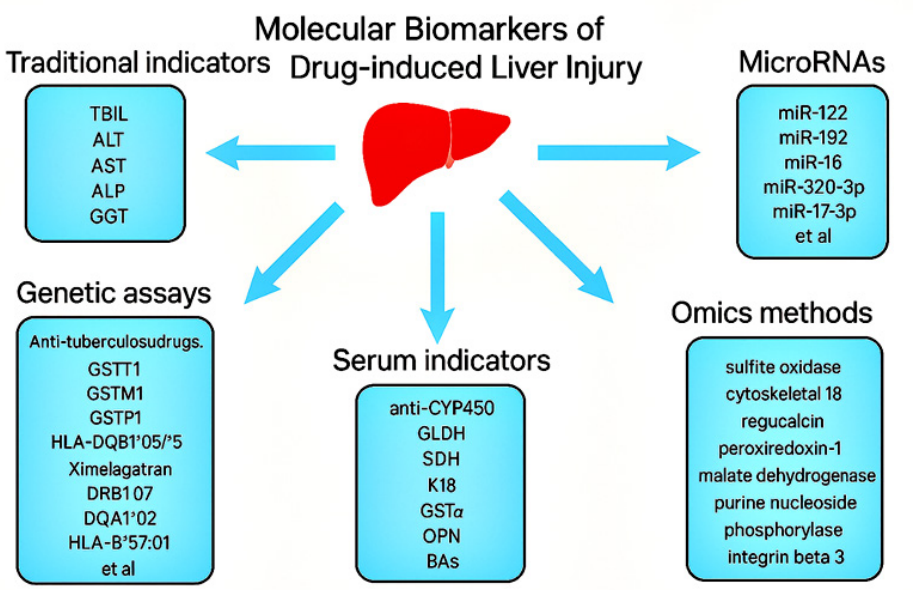

Fig. 3. Diagnostic biomarkers used in hepatotoxicity assessment

Table 4. Catalogue of emerging and omics-based biomarkers in hepatotoxicity

Proposed biomarker	Origin	Specimen	Clinical/Experimental Relevance
	Proteomics		
Interleukin-1, TNF- á	Kupffer cells (major)	Plasma	Hepatic cellular stress
GST-P	Liver cells	Serum	Liver cell damage
Keratin-18	Epithelial cells	Serum	Marker of apoptosis or necrosis
HMGB1	Multiple tissues	Serum	DILI and acute liver failure
Apolipoprotein E	Produced in the liver and many other tissues, including brain and kidney	Serum	Marker of drug-induced liverinjury
	Metabolomics		
Lactate	End product of anaerobic glycolysis	Serum /Plasma	Impaired heapatic clearence
Acylcarnitines	Mitochondrial â-oxidation	Serum /Plasma	Reflect mitochondrial dysfunction and altered lipid metabolism
	Transcriptomics		
lncRNAs (various)	Non-coding RNAs	Liver tissue /Serum	Regulatory roles in liver injury
miRNA-122	Liver specific expression	Plasma	Viral-, alcohol-, and toxin-induced liver injury
miRNA-192	Liver - enriched expression	Plasma	Chemical-inducedliver injury

overdose, with total levels markedly higher in APAP and other hepatotoxicities, indicating that oncotic necrosis predominates.[63-65]Additional proteins such as argininosuccinate synthetase,[66] paraoxonase 1, glutathione S transferase (GST), liver type fatty acid binding protein 1, cadherin 5,[67,68] macrophage colony stimulating factor receptor, and aldolase B are cAMP regulated and show potential as mechanistic markers. While some, such as macrophage colony stimulating factor receptor, have been proposed as inflammatory biomarkers, their role in hepatotoxicity remains under explored.[69]

Emerging biomarkers & omics approaches

Several serum protein–based biomarkers have been explored for assessing liver injury, based on the leakage of hepatocellular proteins into circulation. Most remain experimental and are not yet qualified for routine use. ALT isozymes-ALT1 (mainly hepatic but also in renal and salivary tissue) and ALT2 (present in adrenal cortex, neurons, cardiac and skeletal muscle, and pancreas)—may help localize the source of injury.[70]

Other candidates include sorbitol dehydrogenase, glutamate dehydrogenase (GLDH), serum F protein, GST-alpha, and arginase I.[71]Sorbitol dehydrogenase marks acute hepatic injury in rodents; GLDH is highly liver-specific and unaffected by muscle injury; serum F protein correlates with histopathology in humans[72] but lacks preclinical validation; GST-alpha reflects centrilobular damage but can be induced by xenobiotics; and arginase I rises earliest and most markedly after thioacetamide injury, paralleling ALT/AST.[73,74]

While these markers offer advantages in sensitivity or specificity, their limited validation and overlap with extrahepatic sources constrain their clinical application, and they may be most effective when used in panels with conventional enzymes. Emerging omics techniques provide additional promise: transcriptomics highlights gene-expression changes, proteomics enables broad protein profiling, and metabolomics captures metabolic disturbances, often preceding biochemical alterations. Collectively, these approaches could deliver biomarker panels with greater specificity, sensitivity, and earlier detection potential than current assays.

Transcriptomics has been widely applied in hepatotoxicity research to identify gene expression signatures associated with drug-induced liver injury (DILI). By revealing how hepatotoxins alter gene activation, transcriptomic profiling can uncover predictive patterns such as those linked to hepatic steatosis or oxidative stress pathways, providing early biomarkers and mechanistic insight into liver damage.

Proteomics complements transcriptomics by mapping protein-level changes in response to toxic injury. It highlights alterations in protein abundance and post-translational modifications that reflect hepatocellular stress and damage. As proteins are functional executors of gene expression, proteomic biomarkers help connect molecular changes with phenotypic outcomes, improving the diagnostic value for DILI.

Metabolomics offers a direct view of the hepatocellular state by capturing end products of biochemical pathways. It reveals actual metabolic disruptions during DILI, including mitochondrial dysfunction, bile acid imbalance, and energy metabolism defects. This approach enables earlier detection of injury and supports individualized risk assessment through metabolic phenotyping. [75-76]

Cellular stress markers

The cellular stress response regulates hepatocyte survival or death after toxicant exposure. Proteomic methods such as two-dimensional gel electrophoresis, mass spectrometry and iTRAQ™ have identified stress-related markers in rat hepatocarcinogenesis, including annexins, metabolic enzymes, aflatoxin B aldehyde reductase, and GST-P form. Keratin-18 indicates apoptosis and necrosis, while HMGB1 reflects necrosis and inflammation only. Malate dehydrogenase (MDH), purine nucleoside phosphorylase, and paraoxonase-1 correlate strongly with histopathology in hepatotoxicant-exposed rats; the first is elevated in hepatic and cardiac injury, the second rises early after galactosamine or endotoxin exposure, and the third, an HDL-associated hepatic enzyme, decreases in drug-induced and chronic liver injury. In humans, 92 altered serum proteins were identified in DILI, with apolipoprotein E distinguishing the cases from controls, with 89% accuracy. [77] The catalogue *of emerging and omics-based biomarkers in hepatotoxicity, is given in Table 4.*

CONCLUSION

Biomarkers are indispensable for the timely detection, monitoring, and prognosis of hepatotoxicity. While conventional markers remain clinically valuable, their limitations necessitate the adoption of novel and omics based indicators that can enhance diagnostic specificity and enable earlier intervention. The future of hepatotoxicity assessment lies in integrated biomarker panels supported by high throughput, cost effective technologies and robust validation protocols. Such advancements will not only refine clinical decision making but also improve therapeutic outcomes, ultimately reducing the global burden of liver disease.

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This study did not involve human participants, and therefore, informed consent was not required.

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This research does not involve any clinical trials.

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Not Applicable.

Author Contributions

Shashikala Metri - Wrote the final draft of the work and edited, supervised the entire

work; Rohini Rasamallu & Shaheda Mohammad - Completed all reference work and formatting; Rohini Rasamallu & Shaheda Mohammad - Collected all the data and completed the literature review; Mudunuri Ganga Raju - Provided valuable guidance; Shashikala Metri & Ceema Mathew - Data editing.

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