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Review Article

Exploring Traditional and Novel Diagnostic Biomarkers for Liver Fibrosis: A Comprehensive Review

Yash Subhash Janve[1,*], Jagdish Kakadiya[2]

[1]Department of Pharmacology, Parul Institute of Pharmaceutical Education and Research, Parul University, Gujarat, INDIA.
[2]Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, Gujarat, INDIA.

ABSTRACT

Liver fibrosis is a widespread health issue brought on by a number of causes. A disease's early diagnosis is crucial for improved patient management. Different causes of persistent liver damage can result in hepatic fibrosis. One of the most important steps in determining the disease's severity is determining the degree of liver fibrosis. A disease's early diagnosis is crucial for improved patient management. There are various traditional biomarkers present for diagnosing various stages of liver fibrosis, but these traditional biomarkers have certain limitations. Liver fibrosis having low specificity (ability to determine the etiology) and low sensitivity. In medical practice, test for liver fibrosis have limited usefulness (distinguish intermediate stages). A liver biopsy is a gold standard for evaluating the stage of hepatic fibrosis, despite the fact that it has many disadvantages. Over the past ten years, several noninvasive new markers for identifying the stage of hepatic fibrosis have been created. Some are comparable to liver biopsy and have solid validation. This article aimed that Novel diagnostic biomarkers may prove to be very helpful in the near future for identifying the stages of liver fibrosis and may be just as significant as liver biopsies in doing so.

Keywords: Liver Fibrosis, Traditional and Novel Biomarkers, Biopsy, Collagen.

Correspondence:
Mr. Yash Subhash Janve
Assistant Professor, Department of Pharmacology, Parul Institute of Pharmaceutical Education and Research, Parul University, Gujarat, INDIA.
Email: yash.subhashjanve@gmail.com
ORCID: 0000-0002-1233-9795.

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LIVER FIBROSIS

The liver serves as the body's metabolic center, which is a unique function. Adults weigh about 1400 g on average, with no significant gender differences. It is composed of five main types of cells that account for over 80% of its volume. Extracellular spaces and extracellular matrix elements make up the remaining 20%.

Fibrosis is a frequent liver reaction to a persistent lesion brought on by a variety of aggressors, including congenital defects, viral infections, alcoholism and drug addiction and autoimmune attacks that target hepatocytes and bile ducts.[1-7]

In the Disse region of the normal liver, a structured collection of proteins known as the Extracellular Matrix (ECM) can be seen in close contact with the basal lamina (a low-density substance similar to the basal membrane created by type IV collagen, laminin and entactin along the sinusoidal wall). It supports the parenchymatous cells and makes up roughly 0.5% of the liver's overall weight. Due to the ECM's non-fibrillar structure and ability to sustain the organ's architecture, chemicals can be exchanged between hepatocytes in a semi-continuous flow. This is essential for maintaining all liver cells' specialized tasks.[8]

With the exception of the fact that fibrosis has led to a quantitative enlargement of these elements, the ECM elements in the fibrotic liver are comparable to those found in the healthy liver (collagen and others).

The Kupffer cells' paracrine activation of the hepatic stellate cells causes an overexpression and redistribution of the relative amounts of ECM proteins, changing the sub-endothelial space matrix's typical structure into an interstitial matrix with a high content of fibrillar collagen. These amounts are first deposited in the central vein and/or portal tract, resulting in the creation of fibrous connections between the vascular structures and the eventual loss of the capillarized and microvilli-rich sinusoidal endothelium and hepatocytes. In addition to having an impact on ECM growth, this also prevents the hepatic lobe from undergoing its typical vascularization, which impedes the organ's ability to operate.[9]

These modifications underline the primary role of the ECM in the liver, which involves acting as a continuous network connecting the cells and facilitating continuous signal exchange via its own receptors in addition to serving as a framework for the organ's architecture. Fibrosis is an important biological event in and of itself because it results from an imbalance between the production and breakdown of the ECM components. When associated with

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other liver conditions, it promotes the long-term development of cirrhosis, which in the absence of prompt and effective treatment usually results in mortality.[10]

LIVER FIBROSIS BIOMARKERS

A wide subclass of medical signs, or objective indications of health state viewed from the outside of the patient, which can be quantified precisely and consistently, are referred to as "biomarkers," a portmanteau of "biological marker," in the word.

A biological molecule that can be discovered in tissues, body fluids, or blood and is a symptom of a condition, disease, or a normal or pathological process. To determine how well the body responds to a disease or condition treatment, a biomarker may be utilized. Often known as a signature molecule and a molecular marker.[11]

The diagnosis and management of patients with chronic liver disease depend heavily on the ability to predict hepatic fibrosis. In comparison to the conventional approach of assessing liver fibrosis through biopsy, novel diagnostic biomarkers provide a number of advantages, including safety, cost savings and global accessibility. Hyaluronic acid and tissue inhibitors of metalloproteinase-1 are examples of direct indicators of fibrinogenesis or fibrinolysis that can be included in a contemporary biomarker strategy. Aminotransaminases and platelet count are examples of indirect surrogate indicators of fibrosis. For a number of chronic liver illnesses, including alcoholic and non-alcoholic fatty liver disease, chronic viral hepatitis and hepatitis, several algorithms have recently been demonstrated to be successful. Additionally, it has been shown that a number of models are more accurate than liver biopsy at predicting survival related to the liver and overall survival as well as being dynamic to changes in fibrosis over time.[12]

TRADITIONAL BIOMARKERS

Traditional biomarkers of liver fibrosis typically include markers such as simple liver function test, liver biopsy, hematologic variables and other parameters as shown in Table 1. These indicators, which are frequently raised in liver fibrosis instances, are blood enzymes or proteins that can show liver damage or functioning.[13,14]

SIMPLE LIVER FUNCTION TEST

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels are two basic liver function tests that are associated with liver fibrosis as shown in Table 1 and show inflammation and injury to the liver. Bilirubin levels can also be measured because increased bilirubin might be a sign of poor liver function.[13,14]

Table 1: Types of Biomarkers.

Traditional Biomarkers	
1. Simple Liver Function Test[13,14]	ALT Level-Alanine transaminase, AST Level- Aspartate transaminase, Total Bilirubin Level, Direct Bilirubin Level, Indirect Bilirubin Level, GGT Level- Gamma-glutamyl Transferase, LDH Level- Lactate Dehydrogenase, ALP Level- Alkaline phosphatase, Albumin.
2. Liver Biopsy[13,14]	Ishak score, Metavir score, Knodell score.
3. Hematologic variables[13,14]	Platelet count, Prothrombin times.
4. Others[13,14]	Glucose and Insulin, Apolipoprotein, Haptoglobin, TC Level- Total Cholesterol Level, TG Level- Total Triglyceride Level, LDL Level- Low Density Lipoprotein, HDL Level- High Density Lipoprotein.
Novel Diagnostic Biomarkers	
1. Collagen, ECM molecules and enzymes[13,14]	Procollagen N-terminal peptide, Hyaluronic acid, Type IV collagen, Laminin, YKL-40, Tissue inhibitors of matrix metalloproteinase, MMP-2, MMP-9.
2. Cytokines[13,14]	TGF-β, TNF-α, Angiotensin-II.

3. Proteomic markers[13,14]	Galectin-3 binding protein (G3BP), Microfibrillar-associated protein 4(MFAP-4).
4. Genetic markers[13,14]	SNP of AZIN1, TLR-4, TRPM-5.
5. Combinatorial markers[13,14]	APRI, AST/ALT, Bonacini index, FIB - 4, Fibro index, Fibrometer test, FibroSpect II, Forns test, Hepascore.

ALT Level-Alanine transaminase

The liver cells contain an enzyme called Alanine Aminotransferase (ALT). To make proteins easier for the body to absorb, liver enzymes like ALT break them down. ALT may be produced and released into the blood by a disease affected or inflamed liver. As a result, the ALT concentrations rise. Its normal range is 7 to 55 U/L.

In cases of liver disease, this enzyme's activity may exceed the upper reference limit by 100 times. Peak activities have little bearing on the prognosis and may decrease when the patient's condition deteriorates.[15-17]

AST Level- Aspartate transaminase

The liver cells contain an enzyme called Aspartate Transaminase (AST). This enzyme aids the body's metabolism of amino acids. ALT may be produced and released into the circulation by a disease affected or inflamed liver. The effect is an increase in ALT levels. Its normal range is 8 to 48 U/L.

In cases of liver disease, this enzyme's activity may exceed the upper reference limit by 100 times. Peak activities have little bearing on the prognosis and may decrease when the patient's condition deteriorates.[18,19]

Total Bilirubin Level

When red blood cells are normally broken down, bilirubin is produced. It is a yellowish fluid called bile that is produced by your liver. This liquid aids with meal digestion. Its normal range is 0.1 to 1.2 mg/dL.

The liver's regular activity will remove the majority of the bilirubin from the body. An unhealthy liver may release bilirubin, which then enters the bloodstream. Bilirubin's purpose is to break down red blood cells. Sequential bilirubin monitoring is helpful in determining the severity of liver damage caused by various etiologies. In acute hepatitis, serum bilirubin peaks later than enzymes but remains elevated for a longer period of time.[20,21]

Direct Bilirubin Level

Also known as Conjugated Bilirubin. Indirect bilirubin undergoes covalent modification to become direct bilirubin. This covalent modification is carried out to improve bilirubin solubility and lessen its toxicity. The excretion of bilirubin is facilitated by increasing bilirubin's solubility. The following is how bilirubin and glucuronic acid conjugate. The starting component for the conjugation of bilirubin and glucuronic acid is UDP glucose. Its normal range is less than 0.3 mg/dL.

Liver inflammation can be caused on by a lot of activities, including hepatitis virus infection and excessive drug or alcohol usage. When liver cells are injured by any of the above-mentioned circumstances, the liver may leak both indirect and direct bilirubin into the bloodstream. As a result, levels are boosted.[22,23]

Indirect Bilirubin Level

The first breakdown result of hemoglobin is indirect bilirubin, often known as unconjugated bilirubin. Lipids are able to dissolve indirect bilirubin. It is hence lipophilic. Indirect bilirubin is extremely hydrophobic and insoluble in water. It is simple for indirect bilirubin to pass the plasma membrane. Indirect bilirubin has a significant level of toxicity, particularly to the neurological system. As a result, indirect bilirubin is changed into the conjugated form, which is more soluble and safer. Albumin, the primary protein for bilirubin transport, is connected to indirect albumin. Its normal range is 0.2 to 0.8 mg/dL.

Increased RBC hemolysis (Erythroblastosis fetalis), diseases such sickle cell anemia, hepatitis, cirrhosis and the effects of various medicines, among other factors, can all contribute to an increased level of indirect bilirubin in the serum.[22,24]

GGT Level- Gamma-glutamyl Transferase

The enzyme known as gamma-glutamyl transferase (also known as -glutamyl transferase, GGT, gamma-GT, or gamma-glutamyl transpeptidase) catalyzes the transfer of gamma-glutamyl functional groups from molecules like glutathione to an acceptor, which could be an amino acid, peptide, or water (forming glutamate). GGT is required for the gamma-glutamyl cycle, which is involved in the production and decomposition of glutathione as well as the detoxification of xenobiotics and pharmaceuticals. Its normal range is 7 to 55 U/L.

The levels of gamma glutamyl transferase increase and recover to normal status in liver disorders later than the levels of transaminases. Consequently, the computation of GGT has some value in monitoring the transition from acute to chronic hepatitis when the results stay at high levels. More than any other enzyme,

gamma-glutamyl transferase can grow and remain elevated for extended periods of time-up to twelve times the upper limit of the normal range.[25-27]

LDH Level- Lactate Dehydrogenase

Nearly all living cells contain a lactate dehydrogenase (LDH or LD) enzyme. As it catalases the conversion of NAD+ to NADH and back, LDH also transforms lactate to pyruvate and back. An enzyme called a dehydrogenase moves a hydride from one molecule to another. Its normal range is 105 to 333 U/L.

LDH is crucial in the production of your body's energy. Nearly every tissue in the body, including those in the blood, heart, kidneys, brain and lungs, contains it. In terms of clinical values, LDH is helpful for cancer detection as well as diagnosis or as an indicator for various disorders of the liver and muscles. When examined separately or in combination, the majority of patients had high LDH levels that were linked to one or more forms of LFTs. The accuracy of diagnosing liver illnesses is increased when LDH is used in addition to those liver tests. The ALT-LDH index, which is the other type of LDH, can be used to predict the outcome of acute liver damage in its early stages and to differentiate between acute viral and typhoid hepatitis. By measuring the amount of LDH production in hepatocytes based on a biopsy examination, acute liver failure can also be detected.[13,28,29]

ALP Level- Alkaline phosphatase

The zinc metalloproteinase enzyme, ALP, catalysis the hydrolysis of phosphate esters at an alkaline pH. Whenever there is a biliary tree obstruction, the liver responds by releasing ALP from the canalicular membrane of hepatocytes. Its normal range is 44 to 147 U/L. As a result, the newly created enzyme is absorbed into the bloodstream to increase serum enzyme activity. Elevation typically shows up more in extrahepatic obstruction when compared to intrahepatic blockage. For serum enzyme activity, the upper reference limit may be 10- to 12 times higher. Typically, serum ALP activity in liver illnesses that primarily impact parenchymal cells, including infectious hepatitis, is only moderately elevated or even normal. Increase may also be observed as a result of a drug's therapeutic response.[13,29]

Albumin

The liver has the ability to synthesize enough protein to keep albumin concentrations high until parenchymal damage reaches a 50% level. Its normal range is 3.4 to 5.4 g/dL. Measurements of plasma albumin are 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 content, its usefulness for this purpose is restricted.[29]

LIVER BIOPSY

As shown in Table 1 for the past 50 years, liver biopsy has been regarded as the gold standard for fibrosis classification because it is now possible for doctors to obtain diagnostic information on not only the fibrosis but also other harmful processes like necrosis, inflammation, steatosis, copper and iron deposits, among others. Knodell, Ishak and Metavir are the three grading systems that are most routinely used today to evaluate liver biopsy results.[30]

Ishak Score

The fibrosis was evaluated using the Ishak scale, which has a range of 0 (no fibrosis) to 6 (cirrhosis). This approach was used to document the number of portal tracts, the length and fragmentation of the biopsy. In this strategy, rates of pre-specified clinical outcomes, such as hepatocellular carcinoma and hepatic decompensation, are compared across several Ishak fibrosis stages. The Ishak fibrosis stage can predict clinical outcomes, the need for liver transplantation and patient mortality from liver disease.[31]

Knodell Method

With the publishing of a study on a histologic technique designed to objectively assess abnormalities brought by chronic hepatitis, including fibrosis, lobular necrosis, periportal activity, necrosis and inflammation of the portal vein, in 1981, the Knodell method came into use. It comprises of four numbers that were each given a unique assignment, adding up to a single score The initial component (in periportal and/or bridging necrosis) is scored on a scale of 0 to 10. The following two criteria (intralobular degeneration and portal inflammation) have scores that range from 0 to 4. These three signs add together to show the severity of liver inflammation. The extent of liver scarring is determined by the liver's fourth component, which is classified as F0 (no scarring), F1 (portal fibrosis without septa), F3 (many septa without cirrhosis) and F4 (ample scarring) (cirrhosis or advanced scarring in the liver).[32]

Metavir Scoring Method

The Metavir scoring system was developed primarily to evaluate the condition of the liver in Hepatitis C Virus infected individuals (HCV). The index includes the sum of the scores assigned to the grade of inflammatory activity seen in the sample in addition to the staging score, which represents the degree of fibrosis: 0 (no scarring), 1 (minimal scarring), 2 (scarring has occurred and extends beyond the areas containing blood vessels), 3 (bridges of fibrosis that are extended to and connected with other fibrotic areas) and 4 (severe activity) (cirrhosis).[33]

HEMATOLOGIC VARIABLES

Platelet count and mean platelet volume are two hematological factors associated with liver fibrosis (MPV) as mentioned in Table 1. Due to splenic sequestration, platelet count often decreases in liver fibrosis, while MPV may increase.

Platelet count

Thrombocytopenia, a helpful marker for advanced liver diseases, can be brought on by the emergence of autoimmune processes, myelosuppression brought on by HCV, decreased synthesis of thrombopoietin, hypersplenism and other reasons. However, a significant (70-90%) diagnostic value for fibrosis and cirrhosis is provided by the combined assessment of the AST/ALT ratio and Platelet count (PLT).[32,34]

Prothrombin times

One of the early signs of cirrhosis and fibrosis is the Prothrombin Time (PT), is a gauge of the liver's ability to synthesize substances. Using this PT, Platelet Count and ALT/AST ratio we can predicts the probability of fibrosis as well of cirrhosis with higher perfection.[32,35]

OTHERS

Glucose and Insulin

Hyperinsulinemia and insulin resistance in skeletal and adipose tissues appear to be the pathophysiologic foundations of diabetes in liver disease. Its normal range is 70 to 100g/dL. The blood glucose level will rise as a result. Other contributing factors include a reduced response of the pancreatic islet cells and hepatic insulin resistance. Non-alcoholic fatty liver disease, alcoholic cirrhosis, Chronic Hepatitis C (CHC) and hemochromatosis are more frequently associated with DM. Insulin resistance worsens the failure of the treatment response and hastens the progression of fibrosis in CHC patients. The mortality rate of cirrhotic as well as fibrotic patients is raised by DM.[36]

Apolipoprotein

Hepatocytes are important sites for the production of ApoE, according to research done on rats, marmosets and humans. Along with the liver, the brain, adrenal glands, testicles, skin, kidney, spleen, adipose tissue and macrophages in a various types of tissues are other areas where Production of ApoE is clearly seen. ApoE genotypes are well established to affect plasma lipoprotein levels and to be a major regulator of lipid and lipoprotein metabolism. It was established that the ApoE genotype and the development of Hepatocellular Carcinoma (HCC) in persons who have chronic liver problems for a number of reasons are related. ApoE genotype influences the course of the disease in people who have the Hepatitis B Virus (HBV). Hence, a decrease in ApoE will result in a drop in HDL levels and an increase in LDL levels, which will exacerbate the circumstances of the liver and may lead to hepatic fibrosis.[37,38]

Haptoglobin

The body utilizes the protein haptoglobin, which is generated by the liver, to remove unbound hemoglobin (found outside of red blood cells), from circulation. Haptoglobin attaches to unbound hemoglobin in the blood. The liver quickly removes this haptoglobin-hemoglobin complex from circulation so that it can be broken down and the iron regenerated. Its normal range is 41 to 165 mg/dL. Haptoglobin levels in the blood will temporarily drop when a significant number of RBCs are destroyed because haptoglobin is eaten up more quickly than the liver can generate it. Lower haptoglobin levels are a result of liver disease since liver damage may inhibit both the production of haptoglobin and the clearance of haptoglobin-free hemoglobin complexes.[39]

TC Level- Total Cholesterol Level

Liver disease damages the liver, potentially affecting how well it functions. One of the tasks of the liver is the breakdown of cholesterol. If the liver isn't working properly, the body could start to accumulating cholesterol. Its normal range is less than 200 mg/dL.[12,40]

TG Level-Total Triglyceride Level

Triglyceride molecules are primarily used to store and transport fatty acids in the plasma and cells. The liver is the primary organ involved in the metabolism of fatty acids. By secretion into the plasma or triglyceride-rich Very Low-Density Lipoproteins, fatty acids leave the body. The disturbance of hepatic fatty acid metabolism brought on by obesity and overeating is what causes the clinical condition known as Non-Alcoholic Fatty Liver Disease (NAFLD). Its normal range is less than 150 mg/dL. This condition can progress to Liver Fibrosis.[12,40]

LDL Level-Low Density Lipoprotein

Liver disease damages the liver; it affects the normal functioning of liver. One of the tasks of the liver is the breakdown of cholesterol. If the liver isn't working properly, the body could start to accumulating cholesterol. Its normal range is less than 100mg/dL. So, the level of LDL increases in the body as it produced. Also, in liver disease Apo E decreases which is responsible for production of HDL in body which is produced mainly by Liver.[12,40]

HDL Level-High Density Lipoprotein

The lower HDL cholesterol and greater LDL cholesterol levels in liver illnesses may be due to the decreased synthesis of apolipoproteins A and B. Low amounts of apolipoprotein A-1 have been seen in liver diseases and disorders. So, due to decrease in Apolipoprotein will result in to decrease in to HDL level. Its normal range is greater than 60 mg/dL.[37,38,40]

NOVEL DIAGNOSTIC BIOMARKERS

Novel diagnostic biomarkers for liver fibrosis include collagen, ECM molecules and enzymes as well cytokines, proteomic markers, genetic markers and combinatorial markers as shown in Table 1.[13-14,41]

Collagen, ECM molecules and enzymes

Procollagen N-terminal peptide

During collagen formation, procollagen is enzymatically broken down at the carboxy and amino terminal ends by procollagen C-peptidase and procollagen N-peptidase. Peptides are then released into the serum and their estimations can be used to evaluate matrix deposition. Type I collagen, which produces fibrils, is prevalent in a healthy liver. During fibrogenesis, type I collagen can increase up to eight times. Calculations of serum levels can reveal details about how bad a problem is. A major component of connective tissue is type III collagen, a kind of collagen that generates fibrils. Procollagen III amino peptide concentrations in the basal membrane are greater in hepatic fibrosis brought on by Persistent liver damage (PIIINP). Aminotransferase levels, which indicate the extent of fibrosis in acute hepatitis and PIIINP will be connected. Its normal range is 30-110 mcg/L.[13,41]

Hyaluronic acid

The glycosaminoglycan Hyaluronic Acid (HA), which is a component of the ECM, is produced by the hepatic stellate cells. The Liver Sinusoidal Endothelial Cells (LSECs), which are the structures that conduct this, typically directly affect its absorption and breakdown. People with liver diseases of various causes, notably those with cirrhosis, have been reported to have high levels of HA in their blood; these levels may be due to a decrease in clearance or an increase in production. Its normal range is 0-75 ng/mL. In a study of people with nonalcoholic fatty liver disease, HA was found to be the best fibrosis marker, with an AUROC of 0.97 and specificity and sensitivity of 88-95% and 86-100%, respectively.[32,42]

Type IV collagen

Type IV collagen is essential to the hepatic ECM. In contrast to the proteolytic processing of type I and type III collagens, the existence of this molecule in serum suggests that it has decomposed. This molecule enters the matrix fully. However, in practice, the NC1 and PIVNP tests are more frequently used to identify type IV collagen fragments in serum. They act as sensitive markers for cirrhosis in hemochromatosis and show an excellent correlation with the degree of liver fibrosis in those with chronic viral hepatitis and alcoholic liver disease.[43,44]

Laminin

HSCs produce laminin, a non-collagenous glycoprotein that is deposited in the liver's basal membrane. Laminin builds up surrounding the arteries, in the perisinusoidal area and in the portal triad in hepatic fibrosis. Its normal range is 221 to 1560 pg/mL. Higher serum laminin levels, which are tied to the severity of liver fibrosis and hepatic inflammation, are linked to liver fibrosis regardless of the source.[13,45]

YKL-40

YKL-40 is a glycoprotein (chondrex, human cartilage glycoprotein-39). YKL-40 mRNA is highly expressed in the liver. It is a marker for measuring hepatic fibrosis and has an 80% positive predictive value. It aids in the distinction between liver fibrosis' moderate and extensive stages. Its normal range is 14-155 μg/L.[13,46]

Tissue inhibitors of matrix metalloproteinases

These proteins limit the degradation of the ECM by preventing MMP from carrying out its function. The bulk of MMPs interact with TIMP-1 and MMP-2 specifically interacts with TIMP-2. According to studies, people with cirrhosis have serum levels of TIMP-1 that are 2.4 times higher than those of healthy people. TIMP serum levels rise as the liver disease worsens and are closely correlated with the fibrotic stage.[45]

MMP-2

Increased expression of collagen type I reduces human fibrosis; absence promotes fibrosis; serum marker for alcoholic liver disease. Matrix Metalloproteinase-2 (MMP-2), also known as gelatinase A, promotes ECM remodeling. MMPs of the Membrane Type (MT-MMPs), like MT1-MMP, activate the proenzyme MMP2 after its release. During liver fibrosis, MMP-2 is highly produced in myofibroblasts and is hypothesized to have a profibrogenic effect. Its normal range is 470 to 800 ng/mL.[47]

MMP-9

Leukocytes produce Matrix Metalloproteinase-9 (MMP-9), also known as gelatinase-B, in liver Ischemia and Reperfusion Injury (IRI). MMP-9, a multifunctional metalloproteinase, has an impact on liver regeneration after liver IRI. Promotes HSC apoptosis, is expressed in HCC, increases fulminant liver failure with hepatic encephalopathy, is a serum marker for alcoholic liver disease and is a serum marker for cystic fibrosis. Its normal range is 169 to 705 ng/mL.[47]

Cytokines

TGF-β

Transforming growth factor-1 is the main catalyst for ECM deposition (TGF-1). Through the cell membrane receptors, it exhibits pleiotropic actions. The advancement of hepatic fibrosis

was observed to be correlated with greater TGF- levels in patients with hepatitis C virus infection. A level of less than 75 ng/mL was indicative of stable illness.[45,48]

TNF-α

Tumor Necrosis Factor (TNF-α), an inflammatory cytokine, plays a part in liver inflammation, which over time leads to liver fibrosis. Apoptosis, proliferation and inflammation are all brought on by TNF. Its normal range is 100 to 5000 pg/mL.[49]

Angiotensin-II

An important role in hepatic fibrosis may be played by Ang-II, a vasoactive component of the Renin-Angiotensin System (RAS), which stimulates the production of inflammatory cytokines, mitogenesis, proliferation and collagen synthesis in activated HSCs. Experimental hepatic fibrosis was greatly reduced by Ang-II inhibition or gene deletion. Notably, activated HSCs can produce Ang-II and the primary RAS components are expressed locally in damaged liver tissues.[50]

Proteomic markers

Galectin-3 binding protein (G3BP)

Although the -galactoside binding protein Galectin-3 (Gal-3) has been linked to liver fibrosis, its function in NAFLD is still unclear. by examining the expression of Gal-3 in cells that are native to the liver and any potential links to liver damage. Its examination revealed that many liver cells showed Gal-3. Liver fibrosis was associated with the presence of Gal-3 positive cells and as the disease's severity grew, so did their number.[51]

Microfibrillar-associated protein 4 (MFAP-4)

Microfibrillar-Associated Protein 4 is a collagen-binding protein that has a fibrinogen-like domain at its C-terminus and an integrin-binding motif at its N-terminus. It supports the innate immune response by interacting with the collagen region of surfactant proteins and enables free gaseous exchange in the lungs (SP-A and SP-D). The protein's N-terminal region contains a residual cysteine residue and an Arg Gly-Asp (RGD) sequence, which is a cellular attachment motif for many integrin family members. Among the liver-specific proteins, this protein is a prime marker in serum for the detection of liver fibrosis.[32]

Genetic markers

SNP of AZIN1

Of the several Single Nucleotide Polymorphisms (SNPs) that connect with fibrosis advancement in chronic HCV, an SNP in the Antizyme inhibitor (AzI) gene is the one that is most significantly linked to slow fibrosis progression. The production of a minigene encoding the AZIN1 slow-fibrosis SNP led to a 1.67-fold increase in AZIN1 splice variant 2 (AZIN1 SV2) mRNA in LX2. Human liver tissue in good health has both AZIN1 and AZIN-SV2 mRNAs, while fibrotic liver has decreased amounts of both.[52]

TLR-4

Many liver damages caused by viral hepatitis, alcoholic and non-alcoholic steatohepatitis and cholestatic, autoimmune and drug-induced liver diseases are mediated by TLR4 signaling. Both parenchymal and non-parenchymal cell types in the liver express TLR4. TLR4 causes Liver Fibrosis by activating quiescent HSC into active HSC which results in an increase in ECM production by activating Chemokines and Cytokines.[53]

TRPM-5

The transient receptor potential cation channel subfamily M member 5 (TRPM5) rs886277 polymorphism has been connected to a number of probable causes of liver cirrhosis. The TRPM5 rs886277 polymorphism as a missense (Asn235Ser) variant connected to liver fibrosis in HCV-infected persons was included in the Cirrhosis Risk Score (CRS), which was composed of seven SNPs predictive of fibrosis progression in HCV-infected patients and liver transplantation.[54]

Combinatorial markers

APRI

An index termed APRI is produced by the AST-platelet ratio and is calculated as follows APRI has been demonstrated to be accurate in cohorts of HCV patients and to serve as a stand-in marker of severe liver fibrosis in people with co-infections of HIV and HCV. The APRI can only detect the hepatitis C-fibrosis ratio with a moderate level of precision (63.74%, p 0.01) and with a sensitivity and specificity of 89% and 75%, respectively, according to the findings of a meta-analysis. Recently, it has been utilized to identify advanced fibrosis in HIV patients.[32,55]

AST/ALT

Damaged hepatocytes release these hepatic enzymes into the bloodstream. It has been demonstrated that the AST/ALT ratio can predict non-alcoholic steatohepatitis, chronic viral hepatitis, primary sclerosing cholangitis and primary biliary cirrhosis. In cases of acute, chronic and/or steatosis, this ratio is occasionally less than 1, however, in cases of alcoholic hepatitis, it is commonly larger than 2.[13,32]

Bonacini index

Bonacini cirrhosis discriminant Score formula is as follows-

= Platelet score + ALT : AST ratio score + INR score

As shown in "Table 2" Platelets, ALT/AST ratio and PT are three indicators that show a favourable correlate with histological grades and were used by Bonacini *et al.* to build a discriminant

Table 2: Bonacini Index.

Score	Platelets (10^3/μL)	ALT:AST ratio
0	>340	>1.7
1	280-340	1.2-1.7
2	220-279	0.6-1.19
3	160-219	<0.9
4	100-159	-
5	40-99	-
6	<40	-

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.[56]

score for the diagnosis of severe fibrosis and cirrhosis. This score has a 98% specificity but a 46% sensitivity.[56]

FIB-4

- Fibrosis-4 Index
- Range
- 0-2 mild fibrosis
- 3-4 moderate fibrosis
- 5-6 severe fibrosis

Individuals with any known risk factors for liver disease, including alcoholic liver disease, Non-Alcoholic Fatty Liver Disease (NAFLD), cholestatic and metabolic liver disorders, chronic hepatitis and alcoholic liver disease.

The patient's hepatic fibrosis needs to be watched carefully over time to see whether it is progressing or stabilizing.

A FIB-4 score below that level demonstrated a 90% negative predictive value for advanced fibrosis using a lower cutoff value of 1.45.

On the other hand, if the FIB-4 score was higher than 3.25, advanced fibrosis would be identified with 97% specificity and a 65% positive predictive value.[57]

Fibro Index

Koda *et al.* developed the Fibro index in 2007 to assess hepatic fibrosis in CHC. The results of the platelet count, AST and gamma globulin are used to calculate it. It was associated with F2-F3 fibrosis and had an NPV of 90% at a cutoff value of 2.25. For diagnosing severe fibrosis, this index had an AUC of 0.83; however, subsequent validations showed that it was less trustworthy.[57]

Fibrometer test

The multicomponent FibroMeter (FM) assay measures the patient's age, platelet count, prothrombin index, AST, gamma 2 macroglobulin, HA and ureic nitrogen levels in the blood. FM applicability and performance were validated in the diagnosis of several chronic liver diseases, including chronic HBV and HCV, alcoholic liver disease and nonalcoholic fatty liver disease. One of FM's distinguishing features is how it presents the extent of liver fibrosis as percentages of fibrotic tissue.[57]

FibroSpect II

The Fibrospect II test consists of three parameters: hyaluronic acid, TIMP-1 and 2 macroglobulin. At a cutoff value of 42, it may be able to tell the difference between moderate fibrosis (F0-F1) and severe fibrosis (F2-F4). It was evaluated on CHC patients and the results showed that its AUC for spotting severe fibrosis at phases F2-F4 was 0.831.[45,57]

Forns test

Forns Index is as follows

$$=7\ 811\text{-}3.131\times(\text{In platelet count } [10^9/\text{L}])+0.781\times\text{In}(\text{GT } [\text{IU/L}])3.467\times\text{In (age)}\text{-}0.014\text{cholesterol mg/dL}.$$

Forns *et al.* established this score in 2002 and at a cut-off value of 6.9, it can distinguish between moderate and severe fibrosis based on age, platelet count, serum cholesterol and GT. The results of additional testing on this index showed that it had an AUROC range of 81 to 86%, 94% sensitivity and 51% specificity.[45,57]

Hepascore

Adams *et al.* proposed the Hepascore model in 2005. It combines hyaluronic acid, 2 macroglobulins, serum bilirubin, GGT, age and gender. At a threshold value of 0.5, it showed AUCs of 0.82, 0.9 and 0.89 for the detection of cirrhosis, advanced fibrosis and significant fibrosis in CHC, respectively.[57]

DISCUSSION

For the management of chronic liver disease treatment to be successful, the staging of fibrosis must be done correctly. Noninvasive reproducible tests are necessary to provide the means for disease diagnosis, follow-up and therapy response. It has been difficult for translational hepatology to effectively look for specific biomarkers because of the process of fibrogenesis, which is a common response of the liver to a chronic lesion caused by a variety of aggressions as part of disease progression.

Traditional biomarkers are having certain limitations because of their low specificity (ability to determine the etiology) and low sensitivity. The value of tests for liver fibrosis in clinical practice is limited (distinguish intermediate stages).

Recent studies have focused heavily on non-invasive evaluation of liver fibrosis. Several grades (based on various biochemical indicators or hepatic tissue stiffness) were developed because needle liver biopsy is typically associated with major risks/limitations. The two types of non-invasive serologic testing are direct tests (which, as their name suggests, directly evaluate the metabolism of the extracellular matrix) and indirect tests, which reflect changes in liver function brought on by liver fibrosis. Serological tests may be helpful non-invasive approaches for

identifying liver fibrosis, with a sensitivity and specificity that can reach up to 95%.

CONCLUSION

An ideal biomarker would aid in the diagnosis, monitor illness development and assess how well a treatment is working. The identification of biomarkers may be a simple, easy and economical technique to maintain a watch on the development of liver fibrosis. This creates a sense of urgency in the progression of biomarker identification for liver fibrosis and hepatotoxicity thanks to advancements in the -omics technique.

Novel diagnostic biomarkers can be considered very useful in near future for diagnosing Liver Fibrosis Stages and can be consider equally important as Biopsy in determining Stages of Liver Fibrosis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ECM: Extracellular matrix; **ALT:** Alanine transaminase; **AST:** Aspartate transaminase; **GGT:** Gamma-glutamyl Transferase; **LDH:** Lactate Dehydrogenase; **ALP:** Alkaline Phosphatase; **TC:** Total Cholesterol; **TG:** Total Triglyceride; **LDL:** Low Density Lipoprotein; **HDL:** High Density Lipoprotein; **PIIINP:** Procollagen III amino peptide; **LSECs:** Liver sinusoidal endothelial cells; **HA:** Hyaluronic acid; **HSC:** Hepatic stellate cells; **YLK-40:** Tyrosine, lysine and leucine-40; **TIMP-1:** Tissue inhibitors of matrix metalloproteinases-1; **TIMP-2:** Tissue inhibitors of matrix metalloproteinases-2; **MMP:** Matrix metalloproteinase; **MMP-2:** Matrix metalloproteinase-2; **MMP-9:** Matrix metalloproteinase-9; **TGF-β:** Transforming growth factor-β; **TGF-1:** Transforming growth factor-1; **TNF-α:** Tumor necrosis factor-α; **RAS:** Renin-angiotensin system; **G3BP:** Galectin-3 binding protein; **Gal-3:** Galectin-3; **MFAP-4:** Microfibrillar associated protein 4; **SNP:** Single nucleotide polymorphisms; **AZIN1:** Antizyme inhibitor 1; **TLR-4:** Toll-like receptor 4; **TRPM-5:** Transient receptor potential cation channel subfamily M member 5; **APRI:** The AST-platelet ratio index; **PLT:** Platelet; **FIB-4:** Fibrosis-4 index; **NAFLD:** Non-alcoholic fatty liver disease; **CHC:** Chronic Hepatitis-C; **FM:** Fibro Meter; **HBV:** Hepatitis B Virus; **HCV:** Hepatitis C Virus.

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