Direct Markers of Liver Fibrosis

Amira Maher[1], Ghada M Galal[1], Nagwa Sayed Ahmed[2], El-Zahraa Mohammed Meghezel[1]

Department of Tropical Medicine and Gastroenterology[1], Medical Biochemistry[2],

Sohag Faculty of Medicine, Sohag University.

Abstract

Liver fibrosis is a chronic condition that originates as a result of prolonged hepatic injury. Liver fibrosis is caused mainly by Schistosomiasis, chronic hepatitis C and B viral infections, non-alcoholic fatty liver disease, alcoholic liver disease, cholestatic and autoimmune liver diseases. Hepatic stellate cells are the master cells in the fibrosis process regardless of the cause. HSCs activation involves two separate stages: the initiation stage and the perpetuation stage. The activated HSCs up-regulate gene expression of extracellular matrix elements, matrix-degrading enzymes, and their inhibitors. The components of fibrotic ECM or inflammatory mediators implicated in either the process of fibrosis or degradation of scar tissue could be used as direct markers of fibrosis. Direct indices of fibrosis are classified into direct markers associated with matrix deposition such as HA and laminin, direct markers associated with matrix degradation such as MMPs and TIMPs, and Cytokines and chemokines linked to hepatic fibrosis such as TGF-β1 and PDGF. The validation of these markers for use in clinical practice instead of liver biopsy should be balanced against the possibility of misleading results of these markers as they are affected by factors other than fibrosis.

Keywords: Liver fibrosis, hepatic stellate cell, direct markers of fibrosis

Abbreviations

ALD: alcoholic liver disease**,** CHB: chronic hepatitis B, CHC: chronic hepatitis C, ECM: extracellular matrix, ET: endothelin, HA: hyaluronic acid, HBV: hepatitis B virus, HCV: hepatitis C virus, HSCs: Hepatic stellate cells, KC: Kupffer cells**,** MCP: monocyte chemoattractant protein, MMP: matrix metalloproteinase, NAFLD: non-alcoholic fatty liver disease, NASH: non-alcoholic steatohepatitis, PIIINP: procollagen type III amino-terminal peptide, PCIP: Procollagen type I carboxy-terminal peptide, PDGF: platelet-derived growth factor**,** ROC: receiver operator curve, ROS**:** reactive oxygen species, TGFβ1: transforming growth factor-beta 1**,** WBC: white blood cell**.**

Introduction

Hepatic fibrosis is a chronic process that originates as a result of prolonged hepatic injury. The process of liver fibrosis involves remodeling of hepatic parenchyma with gradual deposition of extracellular matrix

(ECM) and hepatic parenchyma nodular regeneration. When left without treatment, hepatic fibrosis progresses into cirrhosis resulting in progressive liver cell failure [1].

Etiology

Liver fibrosis is caused mainly by Schistosomiasis, chronic hepatitis C (HCV) and B (HBV) viral infections, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), cholestatic and autoimmune liver diseases [2] in addition to other uncommon-diseases like Hemochromatosis and Wilson's disease [3].

Pathogenesis

Hepatic stellate cells (HSCs) are the master cells in the fibrosis process regardless of the cause [4]**.** The process of fibrosis involves the activation of quiescent HSCs to myofibroblast-like cells [5].

HSCs' activation involves two separate stages. The first stage, which is the "initiation phase", involves the initial modifications of HSCs to myofibroblast-like cells to be more reactive with proliferative and fibrogenic cytokines. HSCs' activation is initiated via signals derived from Apoptotic bodies resulting from injured hepatocytes, reactive oxygen species (ROS), and acetaldehyde and lipid peroxidation products. The hepatocyte-apoptotic bodies are phagocytosed by Kupffer cells (KCs). KCs, platelets, polymorph nuclear leucocytes and infiltrating immune cells in addition to sinusoidal endothelium and cholangiocytes produce mediators such as ROS, tumor necrosis factor α, platelet-derived growth factor (PDGF), interleukin-1β, endothelial growth factor, transforming growth factor-beta 1 (TGFβ1) that stimulates HSCs' activation and proliferation [6]**.**

The second stage of HSCs activation (the "perpetuation phase") includes the following changes: survival, migration, proliferation, fibrogenesis. The activated phenotype persists with the persistence of the injurious factor. HSCs' chemotaxis, migration, and proliferation are stimulated by PDGF and the abnormal ECM [6]**.** The process of HSCs' activation is summarized in figure 1.

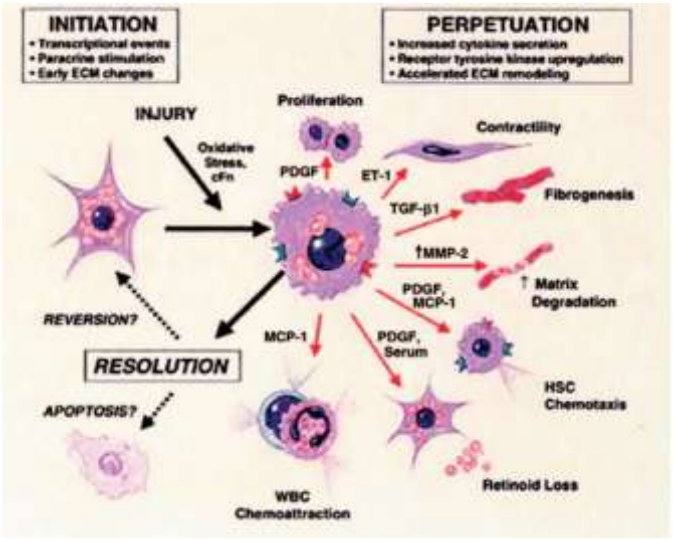

Figure 1: The process of HSCs' activation (Adapted from Hepatic Stellate Cells and Liver Fibrosis) [5]**.**

PDGF: platelet-derived growth factor, ET: endothelin, TGF-β1: transforming growth factor beta1, MMP: matrix metalloproteinase, MCP: monocyte chemoattractant protein, WBC: white blood cell.

ECM remodeling and accumulation occurs as a result of up-regulation of gene expression of ECM elements, matrix-degrading enzymes and their inhibitors by the activated HSCs [5]**.**

The fibrotic ECM contains high amounts of type I collagen [7]**,** matrix glycoproteins such as fibronectin, laminin, and hyaluronic acid [8]**.** The deposition of ECM results from excessive production and decreased degradation of fibrous tissue. In the case of liver damage, the activity of matrix metalloproteinases (MMPs), which are the most potent stimulators of ECM degradation, is suppressed due to the increased production of tissue inhibitors of metalloproteinases (TIMPs) [9]**.**

The components of fibrotic ECM or inflammatory mediators implicated in either the process of fibrosis or degradation of scar tissue could be used as direct markers of fibrosis [9]. These markers are categorized into 1) Direct markers associated with matrix deposition. 2) Direct markers associated with matrix degradation. 3) Cytokines and chemokines associated with hepatic fibrosis [10].

1. **Direct markers associated with matrix deposition:**

- **Hyaluronic acid:**

Hyaluronic acid (HA) was frequently evaluated in chronic hepatitis C (CHC) and NAFLD. It was less frequently evaluated in ALD and chronic hepatitis B (CHB) infection [10]. HA is a valuable test in predicting severe fibrosis [11]. ***Sakugawa et al*** [12] proposed a cutoff value of ≥50 ng/mL for predicting advanced fibrosis. It has a negative predictive value of 98–100 percent for cirrhosis [13]. HA level decreases in CHC patients who are responders to antiviral therapy [14]**.**

- **Laminin:**

Laminin is a noncollagenous glycoprotein that is deposited in the hepatic basement membrane by HSCs [15]. It can predict significant fibrosis in CHC cases with 77 percent accuracy [16].

- **Procollagen type I carboxy-terminal peptide and procollagen type III amino-terminal peptide:**

The Procollagen type I carboxy-terminal peptide (PCICP) is an essential element of the connective tissue [15]. Its upper limit of normal is 202 μg in males and 170 μg in females, and this level increases in cases with moderate to severe liver disease [10].

The procollagen type III amino-terminal peptide (PIIINP) is also an essential element of connective tissue. PIIINP serum values show a correlation with serum bilirubin values in cirrhotic patients. Its upper limit of normal is 0.8 U/mL [17]. In CHC and ALD patients, PIIINP levels increase in patients with more severe liver disease. Moreover, its level decreases in CHC patients who respond to interferon therapy. Furthermore, Serum values of PIIINP are high in cases with autoimmune liver disease and these levels decrease in responders to immunosuppressive therapy [10].

- **Type IV collagen:**

Type IV collagen is an element of ECM with three various areas: an amino-terminal domain (7S domain), a central helix domain, and a carboxy-terminal domain [10]. Type IV collagen 7S domain is considered a direct marker to detect non-alcoholic

steatohepatitis (NASH) and to evaluate the severity of fibrosis in these patients. It has a cutoff value of ≥5 ng/mL for predicting NASH and advanced fibrosis [18].

- **YXL-40 chondrex:**

YXL-40 chondrex is a part of the chitinase family. It has a role in ECM remodeling and degradation [19]. Serum values of more than 330 μg/L are associated with severe fibrosis in various etiologies of liver injury [20].

2. Direct markers associated with matrix degradation:

- **MMPs**: They have a major role in EMC degradation. MMPs have three forms in humans [21]:

a. MMP-1 (collagenases):

Its levels correlate inversely with necrosis and fibrosis. On the other hand, MMP-1/TIMP-1 (tissue inhibitors of matrix metalloproteinases) complex values increase with more severe inflammation, but they have no correlation with the stage of liver fibrosis [10].

b. MMP-2 (gelatinase-A):

MMP-2 is produced by HSCs in diseased liver [10]. In CHC, MMP-2 can predict cirrhosis with an accuracy of 92%. The cutoff value in cirrhosis is 2.4 fold higher than the value in controls [22]. Levels higher than 0.550 are associated with severe fibrosis [23].

c. MMP-9 (gelatinase-B):

MMP-9 is secreted by KCs in the liver [10]. MMP-9 was proposed to have a diagnostic role in hepatocellular carcinoma [24]. MMP-9 has a negative correlation with histological disease in chronic hepatitis, as cirrhotic patients have the lowest serum values [25].

- **TIMPs (TIMP-1, TIMP-2):**

TIMPs are linked to the degradation of collagen [26]. They modify the activation and function of MMPs. The serum value of TIMPs increases with increasing stage of fibrosis [25].

3. Cytokines and chemokines associated with hepatic fibrosis:

- **TGF-β1:**

TGF-β1 is the most potent stimulus for ECM deposition. It has high serum values in the setting of HCV infection and these values increase with increasing stage of fibrosis [27].

- **PDGF:**

PDGF is a major stimulus for HSCs' mitosis in vitro. The level of PDGF increases with increasing stage of fibrosis [28].

Among the above-mentioned markers, HA has the best validation for use in clinical practice [29] and a negative score could be used to preclude fibrosis [30]. HA was extensively evaluated in various forms of liver diseases. The Serum level of HA was found to be correlated with the stage of fibrosis in CHC patients [31-32], both HBeAg positive [33] and negative [34] patients, NAFLD [35], and PBC [36]. Moreover, HA values were raised in ALD patients [37] and high serum HA was a predictor of cirrhosis in these cases [38].

Clinical utility of direct markers of fibrosis

Liver biopsy has many limitations as it is an invasive painful costly maneuver that carries the risk of life-threatening complications such as intra-peritoneal hemorrhage and hemobilia [39-40]. Inter-observer variability [41] and sampling error also may occur [42].

On the other hand, the accuracy of non-invasive markers for evaluation of fibrosis is still unproven. These markers lack specificity to the liver. Moreover, their serum levels are affected by inflammation not only by fibrosis [43]. Furthermore, most of these indices are validated in HCV patients and further research is

required to validate its use in HBV, ALD and other causes of chronic liver diseases [44].

So, in clinical practice, non-invasive markers of fibrosis cannot completely replace liver biopsy [45], and biopsy is still a must in many cases when the values of indirect markers are inconclusive [46].

Conclusion:

Liver fibrosis is a chronic condition that originates as a result of prolonged hepatic injury**.** HSCs have the main role in liver fibrosis regardless of the cause**.** HSCs activation involves two separate stages; the" initiation phase" and the "perpetuation phase"**.** ECM remodeling and accumulation occurs as a result of up-regulation of gene expression of ECM elements, matrix-degrading enzymes and their inhibitors by the activated HSCs. The components of fibrotic ECM, or inflammatory mediators implicated in either the process of fibrosis or degradation of scar tissue could be used as direct markers of fibrosis which are classified into: Direct markers associated with matrix deposition such as HA and laminin, direct markers associated with matrix degradation such as MMPs and TIMPs, and Cytokines and chemokines linked to hepatic fibrosis such as TGF-β1 and PDGF. Among these markers, HA has the best validation for use in clinical practice. However, the clinical validation of these markers for use in clinical practice instead of liver biopsy is still a question. Indirect markers can overcome the invasiveness and other limitations of biopsy. However, this should be balanced against the possibility of misleading results of these markers as they are affected by factors other than fibrosis.

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