# INTRODUCTION

Liver fibrosis is a common pathological consequence of a variety of chronic stimuli including viral, autoimmune, drug induced, cholestatic and metabolic diseases. Advanced liver fibrosis results in cirrhosis, portal hypertension, hepatocellular carcinoma and liver cell failure ***(Elpek, 2014)***.

Chronic liver disease has high global mortality rates. In the World's Largest Hepatitis C Screening Program 29.8 Million persons were screened in Egypt of these, 1.2 million were seropositive (4%) ***(Abdel-Razek et al., 2019).***

Assessment of liver fibrosis help clinicians predict patient prognosis and direct treatment as initiating treatment at early stage of fibrosis is accompanied by higher survival rate ***(Milani and Stasi, 2016).***

The degree of liver fibrosis is determined mainly by liver biopsy, but issues regarding its invasiveness and the small amount of liver tissue evaluated limit its applicability ***(Alves et al., 2017)***. On the other hand, Transient elastography (fibro-scan) is one of the most successful methods for assessment of liver fibrosis due to its noninvasive nature, reproducibility, and high diagnostic performance providing a quantifiable estimate of liver stiffness in units known as kilopascals. However, fibro-scan has some limitations in individuals with narrow intercostal spaces, morbid obesity and increased liver stiffness by causes rather than fibrosis ***(Cequera and García de León Méndez, 2014).***

A series of serum markers of liver fibrosis had been developed. They are classified into direct & indirect markers. Direct markers refer to molecules involved in hepatic fibrogenesis & extracellular matrix turnover such as hyaluronic acid and Procollagen N-terminal peptide, while indirect markers involve molecules that reflect liver function such as ALT, AST and GGT. Direct and indirect markers can be used alone or combined to form composite scores e.g. AST-platelet ratio index (APRI), fibrosis-4 index (FIB-4 index) and hepascore. However, most of these markers are unable of accurate distinction of the fibrosis stage especially early stages ***(Khalili et al., 2014).***

In the past few years, growth differentiation factor 15 (GDF-15) was the matter of research by many scientists. It is a transforming growth factor β (TGF-β) protein related to infection, fibrosis, and apoptosis pathways in case of tissue damage or disease. Its mRNA is known to be found particularly in the liver. In the presence of hypoxia, anoxia, inflammation, radiation exposure, and tissue injuries, the GDF-15 gene is expressed by activated macrophages which increase synthesis of GDF-15 protein ***(Kim et al., 2017)***.

# AIM OF THE WORK

The aim of the present study was to evaluate efficiency of growth differentiation factor 15 in predicting the degree of liver fibrosis in patients with chronic HCV infection through correlation of its levels with fibrosis degree assessed by fibro-scan.

# I. LIVER FIBROSIS

## A. Basic Liver Histology:

The Liver is the largest organ in the body. Hepatocytes account for 60% of total cell population. They are arranged in an anastomosing plate which traverse from portal tract to central vein ***(Boyer et al., 2017).***

Between the plates of hepatocytes are vascular spaces termed sinusoids with large bore fenestrae that allow free exchange of circulating macromolecules with hepatocytes. Basal surface of hepatocytes with the characteristic microvilli is separated from endothelial cell lining sinusoids with space of Disse ***(Burt et al., 2018).***

Classic hepatic lobule is a polygonal structure "hexagon" having terminal hepatic venule "central vein" at its central axis and portal tract at the apices of the hexagon. The region of the lobule around central vein is called centrilobular and the region around portal tract is could periportal ***(Boyer et al., 2017)***.

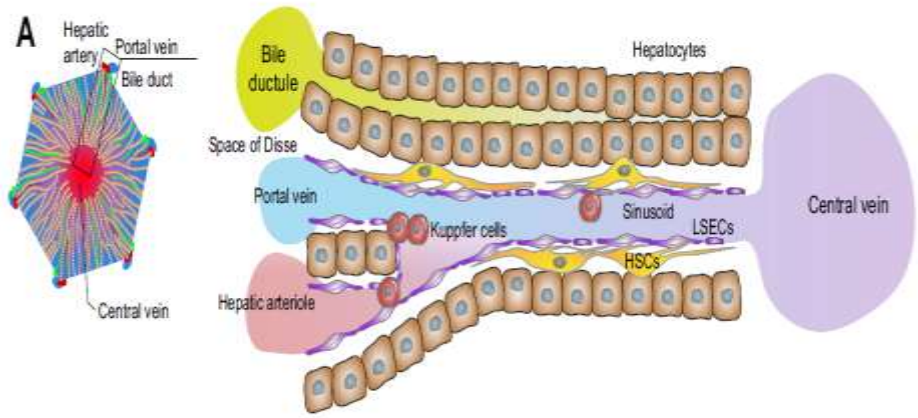


**Figure 1:** Simplified normal liver parenchyma ***(Iwakiri et al., 2014).*** LSECs: Liver sinusoidal endothelial cells; HSCs: Hepatic stellate cells.

The components of hepatic extracellular matrix (ECM) include several families of structural and supporting molecules: collagens, non-collagen glycoproteins, matrix-bound growth factors, glycosaminoglycans, proteoglycans, and matricellular proteins ***(Friedman and Safadi, 2002).***

The ECM of the liver is mainly restricted to liver capsule, portal tracts, sinusoid walls, and central veins ***(Karsdal et al., 2017).***

The most abundant collagens of the liver are; the fibrillar type which includes (I, III, and V collagen), the basement membrane type which includes (IV and XVIII collagen), and microfibrillar type VI collagen. The fibrillar collagens are located around the portal tract and central vein walls, while type IV collagen along with non-fibrillar proteins, laminin and entactin/nidogen, forms a low-density basement membrane-like matrix along the sinusoid walls. This allows for an easy diffusion between blood and liver cells ***(Karsdal et al., 2017).***

## B. Definition of Liver Fibrosis:

Liver fibrosis is the common sequelae of chronic insult to the liver from any etiology, characterized by the replacement of tissue with a collagenous scar. Cirrhosis is the end stage of liver fibrosis resulting in nodules of regenerating hepatocyte surrounded by fibrotic bands and distortion of hepatic vasculature ***(Asrani et al., 2019).***

Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency, portal hypertension, and liver failure ***(Friedman, 2010).*** Indeed, 80 % of all HCC cases develop on an established background of cirrhotic liver disease ***(Kocabayoglu, 2013).***

## C. Epidemiology of Liver Diseases:

According to the Centers for Disease Control and Prevention (CDC), in 2016 there were 4.9 million people living with liver disease. Chronic liver disease and cirrhosis are the sixth leading cause of all-cause mortality in people aged 25–64 years ***(Axley et al., 2019).***

In 2017, the World Health Organization revealed that 71 million people suffer from chronic (HCV) infection worldwide ***(World Health Organization, 2017)***. HCV infection is a major cause of liver cirrhosis and hepatocellular carcinoma (HCC) ***(Ahmed et al., 2019).*** In the World's Largest Hepatitis C

Screening Program 29.8 Million persons were screened in Egypt of these, 1.2 million were seropositive (4%) ***(Abdel-Razek et al., 2019).***

Hepatitis B is one of the most common and severe infectious diseases that leads to significant morbidity and mortality ***(Jefferies et al., 2018)***. Globally, in 2015, an estimated 257 million people were living with chronic HBV infection ***(World Health Organization, 2017).***

## D. Etiology of Liver Diseases:

### 1. Viral Hepatitis:

Hepatitis B and hepatitis C viruses are non-cytopathic viruses. Liver damage is mediated by the host's immune system attempting to clear the virus; virus-specific T cell typically are unable to eliminate the virus with secondary recruitment of mononuclear cells leading to necro-inflammation and chronic infection ***(Böttcher and Pinzani, 2017).***

Some HCV proteins have capability to initiate the activation of pro-fibrogenic and pro-inflammatory properties of hepatic stellate cells (HSCs). HCV proteins induce the production of reactive oxygen species (ROS) and subsequent oxidative stress in hepatocytes, thereby directly contributing to fibrogenesis ***(Böttcher and Pinzani, 2017).***

## 2. Alcoholic Liver Disease (ALD):

Acholic liver disease results from long-standing excessive alcohol consumption. It is characterized by a spectrum of histologic lesions ranging from fatty liver (steatosis) in the majority of excessive drinkers to steatohepatitis and fibrosis in approximately 35%, and approximately 10% progress to cirrhosis ***(Boyer et al., 2017).***

It is well established that ethanol is primarily metabolized in hepatocytes, followed by generation of acetaldehyde and reactive oxygen species (ROS), which cause hepatocellular damage and liver fibrosis ***(Bataller and Gao, 2015).***

Alcohol, its metabolite acetaldehyde, and their byproducts can directly activate HSCs and stimulate collagen I expression ***(Böttcher and Pinzani, 2017)*** and by stimulating immune cells to produce profibrogenic mediators ***(Bataller and Gao, 2015).***

## 3. Non-Alcoholic Fatty Liver Disease:

Nonalcoholic fatty liver disease (NAFLD) is defined as fatty infiltration of the liver exceeding 5% to 10% of liver tissue. It is a spectrum of disorders ranging from simple fatty liver (steatosis without liver in-jury), nonalcoholic steatohepatitis (steatosis with inflammation), and fibrosis/ cirrhosis ***(Ii, 2004).***

Non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are associated with oxidative stress due to formation of ROS induced by a mitochondrial overflow of free fatty acids. This is thought to be a critical factor in fibrosis development ***(Böttcher and Pinzani, 2017).***

**4. Autoimmune Liver Diseases:**

Autoimmune liver diseases (AILDs) include autoimmune hepatitis, primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). They are characterized by loss of immune tolerance to self-antigens leading to hepatic and biliary injury with chronic inflammation & subsequently fibrosis ***(Arndtz and Hirschfield, 2016).***

In primary biliary cholangitis and primary sclerosing cholangitis, intrahepatic accumulation of bile acids contributes to fibrogenesis through induction of hepatocyte and cholangiocyte apoptosis and necrosis ***(Böttcher and Pinzani, 2017)***. Also, cholangiocytes secrete many pro-fibrogenic effectors that have an activating effect on portal myofibroblasts ***(Österreicher et al., 2011).***

**5. Other Uncommon Causes of Liver Fibrosis:**

These include Budd-Chiari Syndrome, Wilson disease cystic fibrosis, alpha-1 antitrypsin deficiency, hemochromatosis, galactosemia and glycogen storage disease ***(Afdhal and Schuppan, 2008).***

## E. Pathogenesis of Liver Fibrosis:

Liver fibrosis is associated with major alterations in both the quantity and composition of ECM. In advanced stages, the liver contains approximately 6 times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans ***(Bataller and Brenner, 2005).*** Pathogenesis of liver fibrosis is discussed in 4 steps "initiation, perpetuation, resolution and fibrosis progression".

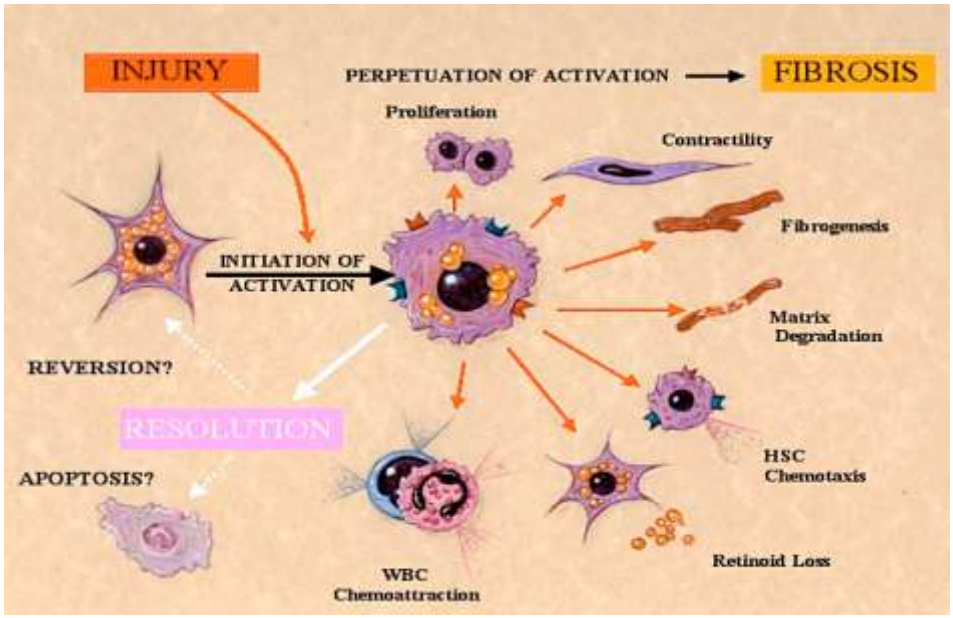


**Figure 2:** Pathogenesis of liver fibrosis ***(Friedman and Reeves, 2002).***

## 1. Initiation:

Activation of hepatic stellate cells (HSCs) remains a central event in fibrosis complemented by other sources of matrix-producing cells, including portal fibroblasts, fibrocytes and bone marrow-derived myofibroblasts ***(Elpek, 2014).***

During liver injury quiescent HSCs become rapidly activated and transdifferentiate into proliferative, contractile and fibrogenic myofibroblast-like cells. Apoptotic bodies derived from damaged or injured hepatocytes and reactive oxygen species (ROS), acetaldehyde and lipid peroxidation products are strong initiating signals for HSC activation ***(Gandhi, 2017).***

Kupffer cells (KC) also phagocytose the hepatocyte-apoptotic bodies. Kupffer cells, when activated, release the pro-inflammatory cytokines TGF-β and TNF-α, and reactive oxygen species, which in turn initiate the activation and proliferation of HSCs ***(Kocabayoglu, 2013).***

Crosstalk exists between sinusoidal endothelial cells and HSCs through the release of fibronectin and endothelin 1 (ET-1) by sinusoidal endothelial cells, which contribute to the conversion of quiescent to activated HSCs ***(Kocabayoglu, 2013).***

Activated HSC (aHSC) adopts a smooth muscle myofibroblast-like phenotype that is characterized by its

expression of smooth muscle alpha-actin "α-SMA" and desmin. The aHSC secretes large quantities of scar-forming type I and III collagens, expresses a variety of other ECM proteins and releases vast amounts of the tissue inhibitor of metalloproteinase 1 (TIMP-1), a protein that prevents degradation of collagen by matrix metalloproteinase ***(Mann and Mann, 2009).***

Platelets are also source of growth factors as platelet derived growth factor (PDGF). TGF beta 1 and epidermal growth factor (EGF). Other sources of paracrine peptide growth factors include lymphocytes and monocytes ***(Friedman and Reeves, 2002).***

2. **Perpetuation**

The second step of HSC activation comprises the many classic features that HSCs display following injury: HSCs proliferate, migrate towards cytokine chemoattractant, contract, degrade matrix, lose their characteristic perinuclear retinoid (vitamin A) droplets, as well as release cytokines and leukocyte chemo-attractants ***(Kocabayoglu, 2013).***

**a. Proliferation:**

The most potent mitogen in HSCs is platelet derived growth factor (PDGF) ***(Elpek, 2014).***

**b. Chemotaxis:**

In healthy liver, HSC reside in the sinusoidal space of Disse. Activated HSC migrates towards cytokine chemo attractants and accumulates at the sites of tissue repair ***(Arriazu et al., 2013)***.

Increased production and activity of cytokines may be critical for both autocrine and paracrine perpetuation of stellate cell activation. Stellate cells amplify the inflammatory response by inducing infiltration of mono- and polymorphonuclear leukocytes ***(Friedman and Safadi, 2002).***

**c. Fibrogenesis:**

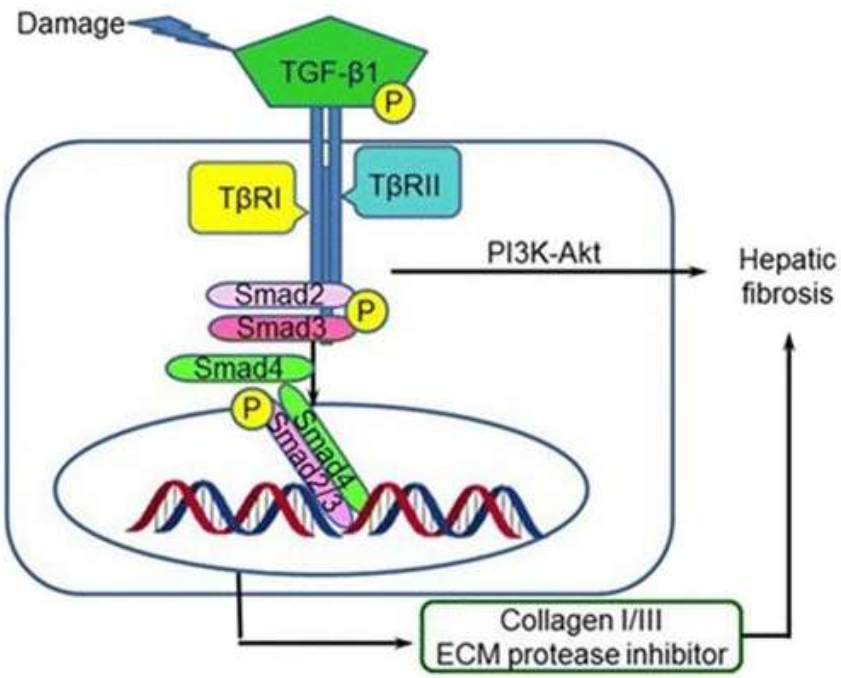


**Figure 3:** Role of TGF beta1in hepatic fibrosis ***(Chen et al., 2015).***

The most potent stimulus to collagen I production is transforming growth factor beta (TGF-beta) ***(Friedman and Safadi, 2002).*** TGF beta1 is present in normal liver, and increases in hepatic fibrosis. TGF-β is produced by Kupffer cells, liver sinusoidal endothelial cells, hepatocytes and HSCs and has paracrine /autocrine effects on HSCs ***(Elpek, 2014).***

TGF β - 1 is stored as an inactivated protein bound to a latency-associated protein. Once activated, TGF-β1 signals via its receptors to Smad proteins, which enhance the transcription of target genes, including procollagen I and procollagen III ***(Friedman and Hernandez-Gea, 2011),*** IV, fibronectin and laminin and accelerates transformation of quiescent stellate cells to myofibroblasts ***(Friedman and Reeves, 2002).*** Upon activation, HSCs lose their characteristic perinuclear retinoid (vitamin A) droplets and acquire a more fibroblastic appearance ***(Friedman and Safadi, 2002).***

TGF beta1 has other profibrogenic effects; In fibroblasts it reduces collagenase and stromelysin gene expression and upregulates the expression of protease inhibitors such as TIMP-1 and plasminogen activator inhibitor, which may protect the matrix from degradation. In addition TGF beta1 increases the mitogenic potency of the principal stellate cell mitogen PDGF-BB ***(Friedman and Reeves, 2002).***

Leptin, a key adipokine, involved in fibrogenesis increases release of TGFb1 from Kupffer cells (***Friedman and Lee, 2011).***

Connective tissue growth factor (CTGF/CCN2) a growth factor-modulator protein secreted from HSCs has a major fibrogenic signal, whose activity can be both TGF beta dependent when produced in hepatocytes, and TGF beta independent when derived from HSCs ***(Friedman and Lee, 2011).***

**d. Contractility:**

Contractility of stellate cells may be a major determinant of increase in portal resistance during liver fibrosis. Activated stellate cells impede portal blood flow by both constricting individual sinusoids and contracting the cirrhotic liver, because the collagenous bands typical of end-stage cirrhosis contain large numbers of activated stellate cells. The major contractile stimulus towards HSCs is endothelin-1 ***(Friedman and Safadi, 2002).***

**e. Matrix degradation**:

Quantitative and qualitative changes in matrix protease activity play an important role in ECM remodeling accompanying fibrosing liver injury. Stellate cells express all of the key components required for pathologic matrix degradation and therefore play a main role not only in matrix production, but also in matrix degradation ***(Friedman and Safadi, 2002).***