

## INTRODUCTION

**H**epatocellular carcinoma (HCC) is a global health problem, although developing countries are disproportionately affected: over 80% of HCCs occur in such regions. Hepatocellular carcinoma (HCC) is strongly associated with either chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and is considered the fifth most common cancer and the third leading cause of cancer death worldwide (*Shariff, 2009*).

There were estimated 748,000 new cases of liver cancer worldwide in 2008, causing 696,000 deaths. Early detection of HCC is therefore extremely important in improving the survival of patients (*Ferlay et al., 2008*).

Alpha-fetoprotein (AFP) has been the only standard serum marker for the detection of HCC for the last 40 years, even though its sensitivity of 39-65% is not very satisfactory, so identification of better early diagnostic biomarkers is crucial (*Shariff, 2009*).

The p53 protein is involved in DNA repair and is an oncoprotective antigen. This gene when damaged, leads to production of anti-p53 and predisposes to various cancers, including HCC (*Di Cesare et al., 2001; Ndububa et al., 2001*).

p53 antibodies are predominantly associated with p53 gene mutations in the sera of patients with various types of cancer. It was reported with high titration in viral-associated HCC Egyptian patients (*Shimada et al., 2003; Atta et al., 2008*).

p53 Abs could be regarded as a specific biomarker for cancer process and its use in combination with AFP may increase the diagnostic sensitivity of HCC (*El-Emshaty et al., 2014*).

## **AIM OF THE WORK**

The aim of this study is to assess the value of measurement of serum level of p53 antibody versus alpha fetoprotein in the diagnosis of HCC in Egyptian patients with liver cirrhosis.

## LIVER CIRRHOSIS

### Introduction

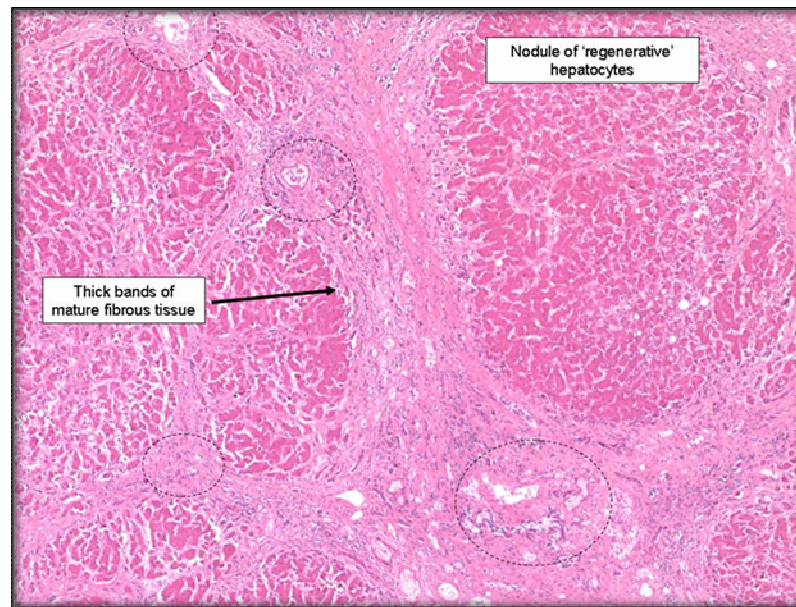
**L**iver cirrhosis represents the final common histologic pathway for a wide variety of chronic liver diseases. The term cirrhosis was first introduced by Laennec in 1826. It is derived from the Greek term *scirrhus* and refers to the orange or tawny surface of the liver seen at autopsy. Cirrhosis is defined histopathologically as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules (**Fig. 1**). The progression of liver injury to cirrhosis may occur over weeks to years (*David et al., 2013*).

It has a variety of clinical manifestations and complications, some of which can be life-threatening. In the past, it has been thought that cirrhosis was never reversible; however, it has become apparent that when the underlying insult that has caused the cirrhosis has been removed, there can be reversal of fibrosis. Fibrosis is defined as an excess deposition of the components of the extracellular matrix (ie, collagens, glycoproteins, proteoglycans) within the liver. This response to liver injury potentially is reversible. In contrast, in most patients, cirrhosis is not a reversible process (*David et al., 2013*).

The World Health Organization (WHO) indicates that 10% of the world population has chronic liver disease; this represents approximately 500 million people with 20 million people worldwide having liver cirrhosis and/or liver cancer. Two million people worldwide die each year from hepatic failure. In the United States the number of discharges with chronic liver disease or cirrhosis as the first-listed diagnosis totaled 112,000 patients in 2007 and 29,165 deaths (9.7 deaths/10000 population) (*Schuppan et al., 2008*).

In the developed countries alcoholic liver disease (ALD), hepatitis C virus infection (HCV) and non alcoholic steatohepatitis (NASH) are the most significant causes of cirrhosis (*Wynn, 2008*).

About one third of patients with chronic HCV who develop hepatic cirrhosis , develop that 15 to 20 years after infection (“rapid fibrotic progressors”), one third develop cirrhosis 20 to 30 years after infection (“intermediate fibrotic progressors”), and one third develop it only after 30 years of HCV infection (“slow fibrotic progressors”) (*Re and kostman, 2005*).



**Figure (1):** Histopathology of cirrhotic liver.

### **Etiology**

The vast majority of patients with liver disease worldwide have chronic viral hepatitis, or steatohepatitis associated with either alcohol or obesity; other etiologies of liver disease include autoimmune attack on hepatocytes or biliary epithelium, metabolic disorders including Wilson's disease, hemochromatosis and a variety of storage disease (*Smit et al., 2008*).

The development of cirrhosis typically requires many years to decades. There are, however, some notable exceptions in which the development of cirrhosis can be greatly accelerated, possibly occurring within months rather than years:

- (1) Neonatal liver disease: infants with biliary atresia may present at birth with severe fibrosis and marked parenchymal distortion.
- (2) HCV infected patients after liver transplantation: a subset of patients who undergo liver transplantation for HCV cirrhosis may develop rapidly progressive cholestasis and recurrent cirrhosis within months, requiring retransplantation.
- (3) Patients with HIV/HCV co-infection: these patients have relatively rapid fibrosis compared to those with HCV alone especially if the HIV is untreated.
- (4) Some cases of drug-induced liver disease
- (5) Severe delta hepatitis.

*(Farci et al., 2004; Smit et al., 2008)*

Once cirrhosis and its complications develop the prognosis is predicted by widely used systems, including Child-Pugh, and MELD which are predictive independent of the etiology of liver disease *(Durand et al., 2008)*.

### **A - Hepatitis C Virus**

The risk and natural history of fibrosis associated with HCV have been greatly clarified as a result of several large clinical studies incorporating standardized assessments of fibrosis that combine detailed historical and clinical information *(Poynard et al., 2001)*.

The disease can run a remarkably variable course, from decades of viremia with little fibrosis to a rapid onset of cirrhosis within 10-15 years. It appears to be host factors rather than viral factors that correlate with fibrosis progression in HCV. The data supporting this conclusion include the following:

- (1) The role of viral factors in fibrosis progression is still debated for the immuno-competent mono-infected patient and multiple studies have shown that viral load or genotype does not appear to have an impact on disease progression even though these former factors affect the response to antiviral therapy (*Missiha et al., 2008*).
- (2) Human promoter polymorphisms (e.g. TGF-beta1 and angiotensin) appear to correlate with fibrosis risk and variation in expression of various mRNA's like of multiple small nuclear polypeptides. SNPs has been shown to impact fibrosis expression and progression to cirrhosis in chronic HCV (*Estrabaud et al., 2012*).
- (3) Host immune phenotype may be critical, as there is more rapid progression in immunosuppressed patients, whether due to HIV or to immunosuppressive drugs (*Missiha et al., 2008*).

**Factors predictive of disease progression:**

Several factors may be important determinants of disease progression in individual patients, including age, ethnic background, gender, HCV-specific cellular immune response, viral diversity, alcohol use, daily use of marijuana, viral coinfection, environmental factors and geography. However, even in a relatively homogeneous population, the outcome is not uniform suggesting that unexplained factors influence the disease (*Wilder and Patel, 2014*)

**Several observations suggest that host factors are important in the progression of chronic hepatitis C:**

- Progressive fibrosis of a number of organs has been associated with production of profibrogenic cytokines. The effect of one of these cytokines, transforming growth factor beta1 (TGF  $\beta$ 1), is enhanced by angiotensin II. They act upon activated hepatic stellate cells which are the major cell type responsible for ECM (extracellular matrix) deposition during liver fibrosis (*Alvarez et al., 2010*).
- Acquisition of HCV infection after the age of 40 to 55 may be associated with a more rapid progression of liver injury (*Ryder et al., 2004*).
- Male sex has been associated with faster fibrosis progression (*Marabita et al., 2011*).

- Co-infection with HIV and Hepatitis B virus (HBV) has been shown to impact progression of disease in chronic HCV. HIV appears to impact both immunity and progression of disease in the setting of HCV where HIV+ individuals have quantitative and qualitative abnormalities of their CD4+ T cells, this results in reduced ability of CD4+ T cells to help viral specific CD8+ T cells making those patients having greater difficulty controlling their HCV viral loads (*Kim et al.,2006*).
- Daily use of marijuana has been associated with development of steatosis and more rapid fibrosis progression, possibly through stimulation of endogenous, hepatic cannabinoid receptors (*Hézode et al., 2008*).
- Alcohol intake: Alcohol promotes the progression of chronic HCV, even in patients with relatively low alcohol intake. Alcohol increases HCV replication, and has also been linked to the acceleration of liver injury (*John-Baptiste et al., 2010*).

Because standard clinical indices cannot distinguish between minimal and even advanced fibrosis, knowledge about these risk factors and duration of infection can greatly inform clinical management. Thus, for chronic HCV, if the time of infection is known and a biopsy obtained at any time thereafter, the rate of progression per year based on either Ishak or METAVIR scoring can be estimated (*Thein et al., 2008*).

Although initial analyses suggested that fibrosis progression is truly linear, it is now increasingly clear that the progression rate accelerates as the disease advances such that it takes less time to progress between Metavir stages 3 and 4, than from stage 1 to 2 (*de Torres et al., 2003*).

## **B - Hepatitis B Virus**

The progression of HBV infection and the evolution of the various phases depends on host genetic factors and molecular characteristics of HBV such as genotype and presence of viral mutations (*Yeung et al., 2011*).

Risk factors for developing cirrhosis include HBV-DNA positive serum levels, an increase in serum transaminase levels, older age, patients with eAg positivity or eAg negative forms, associated with mutations that affect the pre-core region and maintain active viral replication (*Carneiro de Moura et al., 2008*).

In addition, delta hepatitis superinfection or co-infection may greatly accelerate the risk of advanced fibrosis and cirrhosis. What is striking, however, is that virologic suppression in response to potent antiviral regimens can cause remarkable improvement, not only in serum alanine aminotransferase (ALT) levels and histologic inflammation, but also in fibrosis (*Farci et al., 2004*).

The sequelae of chronic HBV infection vary from an inactive carrier state to the development of cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC), extrahepatic manifestations, and death. The prognosis appears to vary with the clinical setting. Long-term follow-up studies of HBsAg positive blood donors have shown that the majority remain asymptomatic with a very low risk of cirrhosis or HCC (*Liaw and Chu, 2009*).

The prognosis is worse in HBV-infected patients from endemic areas and in patients with chronic hepatitis B. The estimated five-year rates of progression are:

- \* Chronic hepatitis to cirrhosis: 12 to 20 percent.
- \* Compensated cirrhosis to hepatic decompensation: 20 to 23 percent.
- \* Compensated cirrhosis to HCC: 6 to 15 percent.

(*Fattovich et al., 2008*)

## C - Alcohol

ALD is one of the leading causes of liver cirrhosis in the Western World. The risk of acquiring alcohol cirrhosis has been suggested to increase when alcohol ingestion increases and in a meta-analysis it has been demonstrated that even a low consumption increases the risk, for 25g/day the estimated relative risk was 1.5-3.6 (*Gunnarsdóttir, 2008*).

Whether there is a threshold of consumption where alcoholic liver disease develops has been studied and it has been estimated that the risk of liver injuries increases when the consumption exceeds 40 g/day. According to different studies the threshold is near 40 g/day, which is 1.1 L of beer, 0.44 L of wine or 0.11 L of spirits (*Savolainen et al., 1993 and Gunnarsdóttir, 2008*).

Another interesting issue is for how long time the high alcohol intake has to exist to develop alcoholic liver disease and it has been estimated to take 16-20 years for men and 10-17 for women (*Gunnarsdóttir, 2008*).

Even though investigators have tried to find a threshold for alcohol consumption and liver injury, only a small proportion of people who have a high alcohol intake will develop liver cirrhosis. In a study from Italy where the average consumption was 60 g/day or more, only 4.2 % had alcoholic liver disease (*Beeentani et al., 1997; Gunnarsdóttir, 2008*).

#### **D - Autoimmune hepatitis:**

Autoimmune hepatitis is a chronic hepatitis that occurs in children and adults of all ages. Its pathogenesis include environmental triggers, a failure of immune tolerance mechanisms, and a genetic predisposition collaborating to induce a T cell-mediated immune attack upon liver antigens, leading to a progressive necro-inflammatory and fibrotic process

in the liver with the presence of circulating autoantibodies and high serum globulin concentrations (*Michael et al., 2010*).

Fibrosis is present in all but the mildest forms of autoimmune hepatitis. Advanced fibrosis connects portal and central areas (bridging), which ultimately, by architectural distortion of the hepatic lobule and the appearance of regenerating nodules, results in cirrhosis. Autoimmune hepatitis can complicate as any progressive liver disease in patients with untreated or unresponsive disease. Primary hepatocellular carcinoma is thought to be a natural consequence of the chronic hepatitis-cirrhosis disease progression. Autoimmune hepatitis is not an exception to this hypothesis, although progression to carcinoma is less frequent than in chronic viral hepatitis (*Yeoman et al., 2008*).

#### **E - Biliary cirrhosis:**

Primary biliary cirrhosis (PBC), a disorder that often affects middle-aged women, is characterized by cholestatic liver enzymes and positive antimitochondrial antibodies. Patients typically develop progressive liver dysfunction resulting in cirrhosis and death if transplantation is not performed. Treatment for primary biliary cirrhosis depends on how early a health care provider diagnoses the disease and whether complications are present. In the early stages of primary biliary cirrhosis, treatment can slow the progression of liver damage to cirrhosis. In the early stages of cirrhosis, the

goals of treatment are to slow the progression of tissue scarring in the liver and prevent complications. As cirrhosis progresses, a person may need additional treatments and hospitalization to manage complications (*Kumagi et al., 2008*).

#### **F - Primary sclerosing cholangitis:**

Primary sclerosing cholangitis (PSC) typically affects young men. It may occur up to 80% of the time with inflammatory bowel disease (especially ulcerative colitis) or as a primary entity. There is no specific serologic marker, and diagnosis is usually made by noting a pruned tree deformity of bile ducts on endoscopic retrograde cholangio-pancreatography or magnetic resonance cholangio-pancreatography (*Lindor, 2007*).

#### **G - Wilson disease:**

Wilson disease (hepatolenticular degeneration) is an autosomal recessive defect in cellular copper transport, with a prevalence of approximately 1 case in 30,000 live births in most populations. An impairment in biliary excretion leads to the accumulation of copper in the liver. Over time the liver is progressively damaged and eventually becomes cirrhotic. (*Roberts and Schilsky, 2008*)

The hepatic injury is believed to be caused by excess copper which acts as a pro-oxidant and promotes the generation of free radicals. Once cirrhosis occurs, copper leaks into the plasma, accumulates in and damages other tissues, and causes