Cirrhosis of liver is defined as a chronic, progressive (?), diffuse process, characterized by fibrosis and structurally abnormal nodules in liver. Cryptogenic cirrhosis means cirrhosis of liver of undetermined etiology. Establishing the etiology of cirrhosis of liver is important, as it indicates specific treatment with its duration, prevents spread of infection to others - vaccination against hepatitis B virus (HBV), the need for familial studies (genetic causes) and the frequency of surveillance (ultrasonography and serum alpha-fetoprotein measurement), to detect asymptomatic hepatocellular carcinoma (HCC). The diagnosis of cryptogenic cirrhosis has dramatically decreased in the 21st century and the reasons for the same are discussed.

Till 1965, cryptogenic cirrhosis was a frequent diagnosis (approximately 50%) as the established causes of cirrhosis were - alcohol abuse (Fig. 1), autoimmune hepatitis, Indian childhood cirrhosis, Wilson's disease, haemochromatosis, primary and secondary biliary cirrhosis, Budd-Chiari syndrome and drug induced. Since then, a series of discoveries in the laboratory and a few clinical observations, have established the etiology of cirrhosis in the vast majority of patients, and the diagnosis of cryptogenic cirrhosis is infrequent (<5%).

In 1965, Baruch Blumberg in Philadelphia (USA), made a Nobel-prize winning discovery of detecting in the serum of an Australian aborigin, the presence of an antigen called Australia Antigen. This was later recognized as the Hepatitis B surface antigen (HBsAg) of HBV. It was soon established that HBV is an important aetiological agent of cirrhosis of liver throughout the world, though its prevalence in the healthy population varies widely (01-15%) in different countries. Subsequently, it was realized that even in the absence of HBsAg in the blood, past HBV infection can be recognized by detecting in the blood – total anti-HBc and/or HBV DNA (occult HBV) or in the liver biopsy – the antigen of HBV on immunohistochemical staining or HBV DNA on polymerase chain reaction (PCR). Another hepatotropic virus – hepatitis delta virus (HDV) was detected in Italy, in the liver biopsy of patients with chronic HBV infection (Rizetto 1977) and the role of two viruses simultaneously damaging the liver and causing cirrhosis of liver was emphasised. Super infection of HDV on chronic HBV infection, rather than co-infection, results in cirrhosis of liver. HDV is totally dependent on HBV for its presence and propagation.

The prevalence of HBV (and HDV) is rapidly decreasing throughout the world – with improved blood bank testing for HBV (radioimmunoassay (RIA) or enzyme linked immunosorbent assay (ELISA) for HBsAg, anti HBc), with the use of disposable syringes – needles, and the protection of the population with a safe - effective vaccine against HBV.

Cirrhosis of liver due to HBV is rapidly decreasing and will continue to decrease.

With the discovery of hepatitis C virus (HCV) in America in 1989, another important viral aetiology of cirrhosis was recognized. The prevalence of HCV varies from 0.1% – 20% (Egypt) in different countries. In USA, HCV is now the most frequent viral aetiology of cirrhosis of liver, an important cause of HCC and the number one indicator for liver transplant. Besides detecting anti-HCV in blood, HCV RNA detection in the blood of immunocompromised subjects (patients in dialysis units, HIV patients, intravenous drug users) has enabled recognition of HCV, as an important cause of cirrhosis of liver. In liver biopsy, HCV RNA can be detected on PCR. With the recognition of HBV (1965), HDV (1977), HCV (1989) role in the aetiology of cirrhosis of liver, the diagnosis of cryptogenic cirrhosis significantly decreased.

For the diagnosis of autoimmune hepatitis, there is no single definitive test and hence some patients with autoimmune hepatitis, were wrongly labelled (in past) as cryptogenic cirrhosis. The four tests for the diagnosis of autoimmune hepatitis have limited positivity: (i) raised gammaglobulin
Table 1: Fatty liver

<table>
<thead>
<tr>
<th></th>
<th>Macrovesicular</th>
<th>Microvesicular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Large droplets pushes nucleus to one side</td>
<td>Small droplets with nucleus in centre</td>
</tr>
<tr>
<td>Causes</td>
<td>Fatty liver of pregnancy</td>
<td>Valproic acid</td>
</tr>
<tr>
<td></td>
<td>Alcohol, Obesity</td>
<td>Tetracycline (parenteral)</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus</td>
<td>Hypertiglyceridaemia</td>
</tr>
<tr>
<td></td>
<td>Hypertiglyceridaemia</td>
<td>Total parenteral nutrition</td>
</tr>
<tr>
<td></td>
<td>Malnutrition, Jejuno-ileal bypass</td>
<td>CT tomography (CT)</td>
</tr>
<tr>
<td>Computed tomography (CT)</td>
<td>Enlarged liver</td>
<td>May not be enlarged</td>
</tr>
<tr>
<td>Mortality</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

(36x568) mortality Low High

(36x579) tomography (CT)

(36x641) (approximately 80%) (ii) antinuclear antibody (60%) (iii) smooth muscle antibody (40%), (iv) liver-kidney microsome −1 (LKM1) antibodies (occasionally). Hence, International Autoimmune Hepatitis Group (IAIHG) included several criteria to calculate a score: for the definite (>15), probable (10-15) and negative (<10) diagnosis of autoimmune hepatitis. For the accurate diagnosis of autoimmune hepatitis, definite criteria on liver biopsy (interface hepatitis, plasma cell infiltration), were also emphasised.

Drug intake history is at times inadequate and an occasional role of a drug (methotrexate, amiodarone, diclofenac, methyldopa, herbal medicines) in the aetiology of cirrhosis of liver should be remembered. The diagnosis of cryptogenic cirrhosis should not be established, unless drug intake history (in past) is rechecked.

Subsequent realization, that non-alcoholic fatty liver disease (NAFLD) is not always a benign condition, but may progress to non-alcoholic steatohepatitis (NASH) and even cirrhosis, resulted in an infrequent (<5%) diagnosis of crypto genic cirrhosis. That NASH causes cirrhosis of liver was not recognized earlier, as fatty liver on liver biopsy is absent, on development of cirrhosis. That patients with a diagnosis of cryptogenic cirrhosis, showed a higher incidence of obesity and diabetes mellitus (type 2) than patients with cirrhosis of known aetiology, indicates that the majority of patients of cryptogenic cirrhosis, were patients of NASH (secondary to obesity and/or diabetes mellitus) who progressed to cirrhosis of liver. Since the prevalence of Metabolic Syndrome X, which includes – abdominal obesity, diabetes mellitus (type 2), hypertension (deadly quartet), is rapidly increasing both in developed and developing countries, the prevalence of fatty liver, NASH and cirrhosis of liver is showing a steady increase, in both adults and adolescents in several countries of the world.

That obesity and/or diabetes mellitus are important causes of cirrhosis of liver is now well established. In fact, after alcohol abuse and HCV infection, NASH progressing to cirrhosis, is the third important cause of cirrhosis of liver in USA. To prevent NAFLD progress to NASH to cirrhosis of liver, obesity, diabetes mellitus and hypertriglyceridaemia should be prevented or detected early and treated effectively in children, adolescents and adults. The major global epidemic of Metabolic syndrome may result in NASH as the number one preventable cause of cirrhosis of liver in near future.

Fatty liver

Fatty liver may be macrovesicular or microvesicular and the differences are shown in Table 1.

Besides alcohol abuse, fatty liver is observed in a wide variety of conditions: obesity, diabetes mellitus (type 2), hypertriglyceridaemia, HCV infection (Genotype 3), congenital apolipoprotein B deficiency (homozgyous or heterozygous), acquired apolipoprotein B deficiency with HCV infection (Genotype 3), Wilson’s disease, malnutrition, total parenteral nutrition, jejunoileal bypass, rapid weight loss, drugs (corticosteroids, oestrogens, tamoxifen, amiodarone).

NAFLD is present in approximately 15% of healthy population, 35% of diabetes mellitus (type 2), 75% of obese and 95% of morbidly obese. NASH is present in approximately 3% of healthy population, 15% of obese and 50% of morbidly obese. NAFLD is a benign condition in the majority of the population but about 10 – 15% of patients progress to NASH (necroinflammation, balloon degeneration, Mallory hyaline, glycogen in nuclei and / or fibrosis). About 15% of patients with NASH, slowly progresses to cirrhosis of liver over 8-10 years. Though most patients with NASH have (asymptomatic) raised blood transaminase values, occasional patient of NASH with normal transaminase value progresses to cirrhosis of liver.

Fatty liver is diagnosed on (i) non-invasive methods (a) Ultrasonography (US) – bright or hyperechoic with normal echotexture; its sensitivity is acceptable but not high. (b) CT without intravenous contrast: homogenous low density - less than 40 Hounsfield unit (HU) or lower compared to that of spleen. (c) Magnetic resonance imaging (MRI) shows fat as bright on T1-weighted images; it is the most sensitive method to detect steatosis in liver or (ii) invasive method - liver biopsy (Fig. 2).

Fibrosis with NASH and Cirrhosis

Fibrosis is a dynamic process with continuous matrix deposition and matrix removal and the possibility of a decrease or disappearance of fibrosis in the liver, has been recently emphasized. Excessive extracellular matrix (ECM) production results in fibrosis. Fibrosis to a large extent is produced by activated hepatic stellate cells (HSC) (Ito cells), present in the space of Disse. Normal HSC produce minimal amount of Type IV collagen while activated HSC produce more Type I and II collagens.

It is important to detect fibrosis in patients with NASH (Fig. 3), as patients with fibrosis (Fig. 4) (rather that with necroinflammation alone), may progress to cirrhosis of liver. In patients with NAFLD, the presence or absence of NASH with or
without fibrosis, should be investigated with liver biopsy and/or non-invasive methods.

Liver Biopsy

Since abnormalities in liver biopsy in nASH, are nearly identical to those seen in alcoholic liver disease, fatty liver is classified as nAFLD or alcoholic fatty liver, depending on the daily intake of alcohol of >20 g/day in the latter group.

To detect fibrosis in patients with nASH, liver biopsy is not advisable in all, as it has a minimal but definite risk of serious complications (haemorrhage, even death in 0.1%).\(^{30,31}\) Though liver biopsy is considered the gold standard, it has some limitations: the size is small (1/50000 of liver), despite adequate tissue sampling error in 10-30%, at times inadequate tissue (less than 15 mm in length and 5 portal tracts), underdiagnosed with macronodular cirrhosis, overdiagnosed with surface biopsy at operation, as capsule extends deeper down causing misinterpretation, and frequent interobserver and occasional intraobserver variations.\(^{27,32-34}\)

Non-invasive Methods

Non-invasive methods (I-VI) are employed, to detect fibrosis in high risk NASH patients: (i) those over 50 years, (ii) with diabetes mellitus, (iii) obese (BMI ≥28 Kg/m\(^2\)), (iv) ALT/AST ratio ≥ 0.8, (v) females (Table 2).\(^{18}\)

1. Indirect Serological Markers
   (i) To detect liver fibrosis, non-invasive indirect serological tests usually performed are – AST/ALT ratio, platelet count and International Normalized Ratio (INR). The various tests are: (a) AST to Platelet Ratio Index (APRI): This test measures AST and platelet count and the ratio is useful to diagnose significant fibrosis and cirrhosis (Metavir ≥ 2, Ishak ≥ 3, Scheuer 3 or 4). The cut off value for significant fibrosis is ≥ 1.5.\(^{36,37}\) (b) Fibrotest measures gamma glutamyl transferase, alpha2 – macroglobulin, haptoglobin, apolipoprotein A-1, and total bilirubin.\(^{38-40}\) It requires a difficult mathematical calculation. Fibrosis is classified in three groups: mild (METAIVIR F0-1), significant fibrosis (METAIVIR F2-4), indeterminate. This test designed to detect fibrosis in liver biopsy of patients with HCV infection is also validated for fibrosis in patients with NASH. (c) Acti test is a modification of the Fibro test and indicates liver fibrosis by AST to indicate necroinflammation activity.\(^{38}\) (d) Hepascore - measures four serum markers – bilirubin, gamma-glutamyltransferase, alpha 2-macroglobulin, hyaluronic acid, and includes age, sex. The cut off value for significant fibrosis is ≥ 0.5.\(^{31}\) (e) Body Mass Index (BMI) ≥28 kg/m\(^2\) (1 point); AST/ALT Ratio ≥ 0.8 (2 points), Diabetes mellitus (1 point). Score ranging from 2-4 has odds ratio of 17 (confidence interval 9.2-31.9) to indicate advanced fibrosis.\(^{35}\) Whether waist circumference indicating abdominal obesity, should replace BMI to improve predictability for fibrosis is not known.

(ii) Retinol-Binding Protein 4 (RBP4)

RBP4 is a vitamin A transport protein in blood which is secreted by adipocytes and stored in liver. In long duration obese adults, serum RBP4 increases with increasing BMI and this adipocytokine promotes insulin resistance (in adults), which is blamed for the development and progress of NASH. In contrast, short duration obese children do not show an increase in serum RBP4 with increasing BMI. In fact, serum RBP4 decreases in children with NASH compared to those with NAFLD, as liver damage and fibrosis increases.\(^{42,43}\)

II. Direct Serological Markers for Fibrosis

(i) The markers of ECM are divided in three groups, indicating: (i) matrix deposition (ii) matrix removal (iii) indeterminate. On the basis of molecular structure, the markers are divided as: (a) collagens, (b) collagenases, (c) glycoproteins, (d) cytokines. The procollagens I C terminal and III N-terminal peptides (PIII N) and transforming growth factor B (TGF-B) in serum indicate matrix deposition. Pro-collagen I and N-peptide and matrix metalloproteinase indicate matrix degradation. Collagenases include metalloproteinases and the tissue inhibitors of metalloproteinases (TIMPS). Cytokines involved in antifibrosis are - interleukin 10 (IL-10) and
interferon-gamma. Assays are available for: PIIINP (RIA), procollagen I (ELISA), Type IV collagen (ELISA, RIA), metalloproteinase (ELISA), TIMP (ELISA), TGF B (ELISA) and glycoprotein hyaluronic acid (RIA, ELISA).

(ii) The direct serological tests for fibrosis are:

Fibrospect II measures – hyaluronic acid, alpha2-macroglobulin, TIMP-I. Maximal sensitivity and specificity are observed only at two extremes of – fibrosis 0 and 4.

European Liver Fibrosis (ELF) Group measures hyaluronic acid, PIIINP, TIMP-I and includes age, to indicate significant fibrosis (Scheuer’s stage 3 or 4 fibrosis).

The combination of indirect and direct serological markers will avoid the necessity of liver biopsy in majority of patients. Since NASH progresses to cirrhosis over several years, only serological tests can be repeated at different time intervals and not liver biopsy. However, in a few patients liver biopsy is essential to judge the presence and stage of fibrosis.

III. Transient Elastography (Fibroscan)

Transient elastography (Fibroscan) requires a costly ultrasonography machine to judge the hepatic tissue stiffness. Fibroscan (Echosens, Paris, France) uses a probe which includes an ultrasonic transducer, a vibration of low (50 MHz) frequency and amplitude, is transmitted into the liver. The velocity of elastic shear wave induced by vibrations is measured by pulse-echo ultrasound and correlates with tissue stiffness. The harder (stiffer) the tissue, the faster the shear wave propagation. Results are expressed as kilopascals (KPa). Fibroscan measures stiffness of an area (1 cm x 2 cm) which is 500 times greater than liver biopsy. KPa of 8.7 correctly diagnosed significant fibrosis (F ≥ 2) and KPa of 14.5 correlated with cirrhosis (F 4). For different aetiologies of cirrhosis of liver, different cut–off values may be employed. Transient elastography, besides accurately assessing liver fibrosis, also predicts presence of oesophageal varices, ascites and HCC. A stiffness value of >13.6 KPa indicates portal hypertension (hepatic venous pressure gradient (HVPG) >10 mmHg) and a value of <19 KPa excludes oesophageal varices.

Though the technique is safe, painless, reproducible, quick, it has serious limitations in the presence of morbid obesity or ascites (as elastic waves do not propagate in liquids). Furthermore, liver stiffness, is also affected by associated steatosis and acute inflammation, besides fibrosis.

IV. Magnetic Resonance Elastography is another method to assess fibrosis in liver and has the advantage of avoiding any sampling error as the whole liver can be studied.

V. Micro-bubble ultrasound is a simple technique to measure hepatic vein transit time which correlates with liver fibrosis.

VI. Breath Tests

13C Caffeine Breath test is of great value to assess hepatic functional reserve but is not of much use to accurately judge fibrosis in liver.

Detailed work up for fibrosis is not required for diseases in which fatty liver does not progress to NASH to cirrhosis. Apolipoprotein B deficiency (heterozygous) usually does not progress to NASH. Fatty liver with malnutrition also does not progress to NASH to cirrhosis, as it is not associated with insulin and leptin (secreted by adipocytes) resistance, observed in patients with obesity.

References


