INTRODUCTION

The epidemic of obesity and associated features of metabolic syndrome including NASH (non-alcoholic steatohepatitis) and NAFLD (non-alcoholic fatty liver disease) is spreading worldwide; thanks to the increasingly sedentary lifestyle. It is estimated that 12-15% of the global population is affected with NAFLD; 50% of diabetics develop NAFLD. It is seen in 57-74% of obese persons and the prevalence rises to 90% in morbidly obese persons. Often the terms - NAFLD and NASH - are used loosely. Hence, there is a need for clear definitions. NAFLD means simply fatty infiltration in absence of alcohol abuse. It encompasses a wide spectrum of conditions associated with accumulation of fat in the liver; ranging from simple steatosis (NAFL) to steatohepatitis (NASH), advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Although NAFL, which is the most common form of NAFLD, appears to follow a benign and non-progressive clinical course, NASH is a potentially serious condition because as many as 25% of patients with the condition may progress to cirrhosis and experience complications of end-stage liver disease. It is therefore not surprising that NASH has been shown to be associated with a high risk of mortality in community population-based studies. The absolute risk of death from NAFLD is however, low with a standardized mortality ratio of 1.34 (95% CI 1.003–1.76, P =0.03) (1). The natural history of NAFLD over a decade is depicted in figure 1.3,4

ETIOLOGY

The common causes of NAFLD include obesity, type II diabetes mellitus, hyperlipidemia, medications, insulin resistance and metabolic syndrome. Other conditions that may also lead to NAFLD include intestinal bypass, malnutrition, starvation, rapid weight loss, total parenteral nutrition, Wilson’s disease, galactosemia, fever and systemic diseases.

Drugs that are known to cause fatty liver include glucocorticoids, synthetic estrogens, aspirin, calcium-channel blockers, tamoxifen, methotrexate, tetracycline, valproic acid, cocaine, zidovudine, didanosine, antimony, bleomycin, coumadin and barium salts.

PATHOGENESIS

The predominant feature of NAFLD is insulin resistance (IR). Insulin resistance is the underlying common feature between NAFLD and metabolic syndrome which is defined as visceral obesity, hypertension, IR or diabetes, and dyslipidemia. Insulin resistance and hyperinsulinemia lead to an increased synthesis of glycogen, lipids and proteins. The exact mechanisms of such actions are not fully understood. However, the prevailing “two hit” hypothesis constitutes the most widely accepted pathogenetic mechanism for the development of NAFL and NASH. The “first hit” is due to the increased influx of free fatty acids into the liver from visceral adipose tissue via the portal vein. It stimulates triglyceride (TG) synthesis in hepatocytes and causes a decrease in β-oxidation, which in turn leads to the development of NAFL. The “second hit” includes all mechanisms contributing to the development of intrahepatic necroinflammation and fibrosis. It is suggested that NAFL progresses to NASH when adaptive mechanisms that protect hepatocytes from fatty acid-mediated

Steatosis
1-20%

NASH and Fibrosis
5-30%

Advanced fibrosis
2-30%

Cirrhosis
25%

Hepatocellular cancer
33%

Liver related death

Fig. 1: Natural course of NAFLD over 8-13 years.
lipotoxicity become overwhelmed and rates of hepatocyte death begin to outstrip mechanisms that normally regenerate dead hepatocytes. The myofibroblasts generate excessive matrix and produce factors that stimulate expansion of liver progenitor populations. The progenitor cells produce chemokines to attract various kinds of inflammatory cells to the liver. They also differentiate to replace the dead hepatocytes. The intensity of these repair responses generally parallels the degree of hepatocyte death, resulting in variable distortion of the hepatic architecture with fibrosis, infiltrating immune cells, and regenerating epithelial nodules.

**EVALUATION**

Patients may either be asymptomatic or present with pain or discomfort in right hypochondrium, smooth hepatomegaly and features of underlying diseases (vide supra). Investigations may reveal raised serum ALT and bright echogenic liver on ultrasonography.

A number of diseases need be excluded in patients with NAFLD, namely alcoholic liver diseases, chronic hepatitis B, chronic hepatitis C, hypothyroidism, autoimmune liver diseases, Wilson's diseases and hemochromatosis. Pertinent blood and imaging tests should therefore be done. Alcoholic steatohepatitis (ASH) constitutes an important and common differential diagnosis. It is identified by a history of alcohol consumption (unfortunately often patients conceal this) and an AST/ALT ratio > 2 (in contrast, ASH and AST/ALT ratio < 1 in NASH).

**HISTOPATHOLOGY**

The definitive diagnosis however, requires a histological confirmation and hence liver biopsy is considered essential.7,8 Liver histopathology in NASH may show, steatosis, hepatocyte ballooning degeneration, intrahepatic inflammation, perivenular and perisinusoidal collagen deposition, Mallory's hyaline body, bridging septa and cirrhosis. These changes are graded and staged as below (see Fig. 2).

**GRADING OF NAFLD**

Grade 1 (Mild): Steatosis predominantly macrovesicular involving 33-66% hepatic lobules.

Grade 2 (Moderate): Any degree of steatosis; usually mixed, both macrovesicular and microvesicular.

Grade 3 (Severe): Mixed steatosis (usually 66%) with florid steatohepatitis.

**STAGING OF NAFLD**

Stage 1: Zone 3 perivenular, perisinusoidal or pericellular focal or extensive fibrosis.

Stage 2: Stage 1 plus focal or extensive portal fibrosis.

Stage 3: Bridging fibrosis; focal or extensive.

Stage 4: Cirrhosis with or without residual perisinusoidal fibrosis.

**NONINVASIVE MARKERS OF NAFLD**

Since liver biopsy is an invasive procedure and may be associated with complications such as bleeding as also with sampling error ie missing out the disease because of patchy involvement, search has been on for noninvasive markers of disease severity. Several such markers have indeed been identified and a few of them have been validated prospectively. The most important feature of a diagnostic non-invasive marker is a high sensitivity and specificity, providing a value as near to 1 of area under the receiver—operating characteristics curve (ROC). This area ranges between 0.5 (no prediction) to 1.0 (perfect prediction). Other ideal features include reproducibility, close correlation with disease severity and clinical outcomes. Also the results of these markers should not be influenced by drugs and extrahepatic diseases. Noninvasive markers for NAFLD can be divided into two broad categories:

1) Imaging techniques; and 2) biologically based markers.

**IMAGING TECHNIQUES**

Fatty deposition and advanced fibrosis/cirrhosis can be easily demonstrated with ultrasonography, computerized tomography or magnetic resonance imaging but the difficulty appears in picking up early fibrosis.

**Transient hepatic elastography (TE)** is a novel technique that has been developed recently and that is capable of detecting fibrosis in early stages.9 It uses an ultrasound transducer probe mounted on the axis of a vibrator which transmits vibrations of mild amplitude and low frequency into the tissues via the transducer, thereby inducing an elastic shear wave that propagates through the tissue. Pulse-echo ultrasound acquisitions are also performed to track the propagation of the shear wave and to measure its velocity which varies according to tissue stiffness. The stiffer the tissue, the faster the shear wave propagates. The results are expressed in kilopascals (kPa). Measurements are taken in the right lobe of the liver through the intercostal spaces with the patient lying in dorsal decubitus, with the right arm at maximum abduction. The tip of the transducer probe is covered with coupling gel and placed on the skin between the ribs at the level of the right lobe of the liver. The operator, assisted by ultrasound time—motion images, locates a portion of the liver that is at least 6-cm thick and free of large vascular structures. Once the measurement area has been located, the operator presses the probe button to begin below the skin surface. Acquisitions lacking the correct vibration profiles or correct follow-up of the vibration propagation are automatically thrown out by the software. The success rate is calculated as the ratio of the number of successful measurements in relation to the total number of acquisitions. The median value of successful acquisitions is then retained as representative of liver stiffness and is called the ‘LSM’. Only LSMs obtained with at least five successful acquisitions and a success rate of at least 50% are considered reliable. The median value of all acquisitions is considered to represent the liver elastic module for each
patient. Advanced liver fibrosis is defined as a median liver stiffness greater than 9.5 kPa. Yoneda et al. recently reported that transient elastography documented progressive increase in liver stiffness along the stages of fibrosis and had an excellent sensitivity and specificity in identifying cirrhosis in patients with NAFLD. A major limitation of the test in patients with NAFLD is presence of obesity wherein a BMI of more than 28 is independently associated with failure of TE examination. Additionally, presence of congestive heart failure may influence measurement of liver stiffness.

SPECT (Single photon emission computed tomography) and in vivo phosphorus 31 magnetic resonance spectroscopy (MRS) are other evolving modalities to assess severity of fibrosis in patients with chronic liver disease. Using minimum spleen pixel count and maximum right hepatic lobe pixel count, SPECT can identify fibrosis in a fairly early stage. However, more experience is needed for wider usage of these techniques in clinical setting.

**BIOCHEMICAL MARKERS**

There are direct and indirect serological markers of fibrosis. They are listed in Table 1 and 2, respectively.

Using varying combinations of indirect markers scoring systems have been developed and evaluated to estimate extent of liver fibrosis and its aggravating factors, steatosis and NASH. These tests include, FibroTest™, SteatoTest™ and NashTest™, respectively. FibroMax™ (Biopredictive, Paris, France) is the association of these three tests on the same result sheet and provides physicians with simultaneous and complete estimation of the liver injury associated with NAFLD. Details of these are beyond the scope of this write up and hence the reader is referred to a review.

**MANAGEMENT**

The mainstay of treatment of NAFLD is aimed at improving the underlying peripheral and hepatic insulin resistance. Dietary change, exercise, weight loss, and pharmacotherapy may all improve insulin resistance and are thus targets for therapeutic trials. Additionally, agents that reduce oxidative stress and/or apoptosis or have cytoprotective properties have been evaluated. Simultaneously, specific underlying components of the metabolic syndrome (MS) such as hypertension, diabetes mellitus, or dyslipidemia should also be treated.

**LIFESTYLE MODIFICATIONS**

Since the main reasons for the dramatic increase in NAFLD have been increased consumption of refined carbohydrates and saturated fatty acids along with decreased intake of milk and mono- and polyunsaturated fatty acids, dietary intervention and exercise emerge as the first line therapy for this disease. Even though they lack appeal and are often limited by patient compliance, diet-induced weight loss is associated with physiologic changes that result in improved insulin sensitivity, reduced adipose tissue inflammation and reduced hepatic free fatty acid supply. Two preliminary studies do show improved serum aminotransferases with weight loss. However, appropriately powered, prospective, randomized controlled trials of dietary intervention and weight loss in patients with NASH are lacking, and that is why it is difficult.
to make evidence-based recommendations for their use in NAFLD. A commonsense approach may thus be to aim for a target weight loss of 7-10% of the baseline weight in 6-12 months in patients with a BMI greater than 25; this reduces aminotransferase levels and diminishes hepatomegaly.\textsuperscript{14}

Weight loss through diet and exercise should be gradual. Rapid weight loss exceeding 1.6 kg/wk has the potential to worsen steatohepatitis and cause gallstones.\textsuperscript{15}

Mono and poly-unsaturated fats may potentially improve insulin resistance and may be beneficial in improving hepatic steatosis.\textsuperscript{16}

**PHARMACOTHERAPY**

Potential pharmacotherapeutic approaches include therapies directed at improving insulin resistance, reducing oxidative stress, decreasing hepatic fibrosis, improving the underlying metabolic syndrome, or promoting weight loss. Surgical approaches directed at weight loss have also been investigated.

**METFORMIN**

Metformin, commonly used to treat diabetes mellitus, improves blood glucose levels by decreasing hepatic glucose production and increasing glucose utilization in peripheral skeletal muscle.\textsuperscript{17}

In the treatment of NAFLD, however, it has produced mixed and generally disappointing results.

Marchesini et al\textsuperscript{18} treated 20 biopsy-proven NASH patients with metformin 500 mg 3 times per day for 4 months. Significant improvement was noted in insulin resistance and aminotransferase levels, with 50% of subjects normalizing their transaminases. Liver volume, as measured by ultrasound, decreased by 20%. Subsequently, Bugianesi et al\textsuperscript{19} followed 110 non-diabetic patients with NAFLD over a 12-month period: 55 were treated with metformin 2000 mg daily, 28 with vitamin E, and 27 with a prescribed diet. Aminotransferases improved in association with weight loss in all groups with metformin-treated patients showing the most biochemical improvement in multivariate analysis. Posttreatment liver biopsy performed on 17 of the metformin-treated group showed statistically significant changes in liver fat, necroinflammation, and fibrosis. Indeed, a recent meta-analysis published in Cochrane database showed that metformin leads to normalization of serum aminotransferases in a significantly greater proportion of patients compared with dietary modification (odds ratio: 2.83; 95% CI: 1.27-6.31) and improved steatosis by imaging (odds ratio: 5.25, 95% CI:1.09-25.21).\textsuperscript{20}

**THIAZOLIDINEDIONES**

The thiazolidinediones (TZDs) are another class of diabetic medications that have been well studied in the treatment of NASH. These medications include pioglitazone and rosiglitazone, which act as peroxisomal proliferator activated receptor agonists leading to increased fatty acid oxidation and decreased fatty acid synthesis within hepatocytes. The resultant improved insulin sensitivity in both hepatocytes and skeletal muscle is one mechanism of action that may explain TZD’s usefulness in NASH patient populations. Several studies have appeared but the latest of them, FLIRT trial, probably depicts the limited benefit from TZD treatment of NASH. In this study, 63 patients with NASH were treated for one year with either rosiglitazone or placebo.\textsuperscript{21} The patients receiving rosiglitazone showed a statistically significant improvement in serum aminotransferases, insulin sensitivity, and hepatic steatosis, but they did not show an improvement in other histologic parameters, notably the NAFLD Activity Score (NAS) or fibrosis.

The side effects of the TZDs may also limit substantially the usefulness of these medications. They include weight gain, pedal edema (seen in up to 5% of patients), osteoporosis and an increase in adverse cardiac events.\textsuperscript{22}

**STATINS**

Although statins appear safe for the treatment of hyperlipidemia in NAFLD patients, the issue of whether or not they are efficacious in the treatment of NASH is unclear. Two small pilot trials have shown improvement of liver enzymes with atorvastatin.\textsuperscript{23,24} Pravastatin 20 mg given for six months normalized liver enzymes and improved hepatic inflammation in five patients with NASH.\textsuperscript{25} Clearly, larger placebo controlled studies are needed to substantiate these results.

**CYTOPROTECTIVE MEDICATIONS**

Ursodeoxycholic acid (UDCA) is a common hepatoprotective agent used and preliminary clinical studies showed some benefit. However, a large multicenter trial with 107 NASH patients treated with UDCA or placebo for 2 years.\textsuperscript{26} Although there was a significant histological improvement in the posttreatment biopsies of the UDCA group, comparable findings were present in the placebo group, with an overall 40% reduction in steatosis and 21% reduction in fibrosis. This study highlights the need for large placebo controlled trials to confirm if UDCA does indeed benefit. UDCA may well be useful in combination with other treatments and hence that aspect also needs exploration.

Betaine is another medication which increases levels of S-adenosyl-L-methionine (SAM), a known essential player in cellular membrane integrity and hepatoprotection, and has been investigated in the treatment of NASH. One year of treatment with betaine in 10 NASH patients significantly improved serum transaminases as well as hepatic steatosis, inflammation, and fibrosis.\textsuperscript{27} However, larger trials are needed to prove the value of betaine in treating NAFLD.

**ANTIOXIDANTS**

A beneficial role of vitamin E in NASH has been long suspected but not proven. In a recent randomized, placebo-controlled study a combination of vitamin E and vitamin C was studied in 45 patients with NASH.\textsuperscript{28} Although serum aminotransferases and necroinflammation were not improved after 6 months of therapy,
repeat biopsies showed significant improvement in fibrosis scores between the two groups. This study needs to be replicated and other antioxidants should be studied.

WEIGHT LOSS PROMOTION

As a part of lifestyle modification, weight loss is expected and that has shown benefit in NAFLD patients as mentioned above. Thus, use of medications and surgical interventions to reduce weight have also been evaluated.

ORLISTAT

Orlistat is a well recognised and frequently used weight loss medication. It inhibits gastric and pancreatic lipase, which is needed to break down triglycerides into free fatty acids and has been shown to prevent 30% of dietary triglycerides from being absorbed. Pilot studies with orlistat given for 6 months along with dietary counselling have shown significant improvement in serum aminotransferases as well as hepatic steatosis and inflammation.29

Two subsequent randomized placebo controlled trials substantiated the above findings and suggested that improvement in NAFLD patients may occur even with modest weight loss.30,31

RIMONABANT

The endocannabinoid (EC) system is involved in the regulation of food intake and body weight. In the setting of obesity, the EC system appears to be upregulated and as such, represents a novel target for medical therapy of NASH. The EC system encompasses a network of receptors and their ligands as well as the enzymes that synthesize and degrade these ligands.32 The cannabinoid type 1 (CB1) receptors are found throughout the body and their activation leads to increased hepatic lipogenesis, fatty acid synthesis in adipocytes, and decreased adiponectin.

Rimonabant is a selective CB1 receptor antagonist that has been shown to decrease hepatic lipogenesis and increase satiety, adiponectin levels, and glucose uptake, thereby improving insulin levels and lipid profiles.33,34 Two large randomized placebo controlled multicenter trials have shown the efficacy of this agent in causing substantial weight loss along with an improvement in the metabolic profile, particularly the lipid profile (decrease in low-density lipoproteins and triglycerides) and improvement in insulin sensitivity with resolution of metabolic syndrome in one-third of patients. These metabolic benefits appear to exceed what could be attributed directly to the weight loss and suggest direct effects of this medication, which may have application in the treatment of NASH.

Two large multicenter, randomized, placebo controlled trials (one in diabetic patients and one in nondiabetic patients) are indeed underway currently to assess any role of this medication in the treatment of NASH. One potential side effect of the drug that requires further evaluation is an association with psychiatric problems, particularly depression.

SURGICAL INTERVENTIONS

In case medical therapy fails, one has to resort to some form of bariatric surgery (Roux-en-Y gastric bypass operation or laparoscopic adjustable band gastroplasty). Numerous studies using different techniques have demonstrated significant improvement in steatosis, hepatocellular injury, and fibrosis. In fact, complete resolution of NASH in as many as 75 to 100% of cases has been described in some series.35 However, not all results are so positive, and collective evidence suggests that a small minority of bariatric surgery patients may develop either worsening steatohepatitis or mild fibrosis.

CONCLUSION

Sedentary lifestyle and increased energy intake in the form of refined carbohydrates have led to a virtual epidemic of NAFLD. In the subset of these patients with NASH, accumulation of fat in hepatocytes with subsequent oxidative stress and inflammation can lead to fibrosis, which may ultimately progress to cirrhosis or promote the development of HCC. Treatment strategies ranging from simple lifestyle modifications to pharmacologic agents and even invasive surgical procedures have shown promise in arresting the progression of this chronic liver disease. They may however, be associated with certain complications. That is why prevention is the best option and that is comprised of 'healthy lifestyle' – low carbohydrate, high fibre diet, regular exercise and a good control of diabetes, hyperlipidemia, hypertension and hypothyroidism, if present.

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