Nonalcoholic Fatty Liver Disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) has a spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis liver and even hepatocellular carcinoma. NAFLD is increasing day by day because of the recent epidemic of obesity, diabetes and hyperlipidemia. Insulin resistance is thought to be the main factor, which causes the increased fatty acid load to the liver, causing steatosis. Increased oxidative stress due to increased mitochondrial oxidation causes further injury by induction of cytokines, lipid peroxidation and fas ligands and progression from a stage of steatosis to steatohepatitis. Diagnosis of NASH can either be presumptive based on clinical and laboratory grounds by excluding other causes of chronic liver disease or definite by way of liver biopsy. Pharmacological treatment of patient with NAFLD and NASH is still evolving. Weight reduction and exercise, which improve insulin remains the first-line of treatment.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a broader term with a spectrum including patients with simple steatosis, steatohepatitis that can progress to cirrhosis liver and even hepatocellular carcinoma (Figure 1).[1,2] Non-alcoholic steatohepatitis (NASH) is believed to be an intermediate stage of liver damage and is a recently recognized entity,[3] which histologically simulates alcoholic hepatitis in the absence of significant alcohol intake.[4] In contrast to patients with simple steatosis, the course in NASH can be progressive and can lead to cirrhosis and hepatocellular carcinoma and thus require therapeutic interventions.

NASH has gained more clinical importance recently because it was suggested to be a major cause of cryptogenic cirrhosis[5] and unexplained raised transaminases[6] and can even lead to hepatocellular carcinoma.[2] Even in the absence of significant alcohol intake, the liver histology in these patients is indistinguishable from alcoholic hepatitis and the term "NASH" was introduced by Ludwig et al to describe this entity.[3]

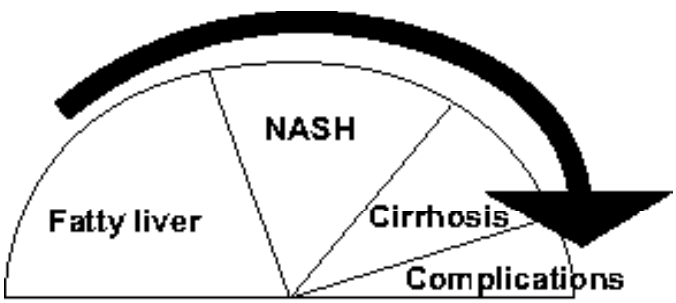

Fig. 1 : Spectrum of Nonalcoholic Fatty Liver Disease

EPIDEMIOLOGY OF NASH

NAFLD can occur at all ages, even though the highest prevalence is described in those between 40-50 years of age.[7] In 1980, Ludwig et al[3] described the term NASH as a form of liver injury that was histologically consistent with alcoholic hepatitis but occurred in obese, diabetic females, who denied alcohol use. However, subsequently it became clear that NASH has an equal sex distribution and many patients are neither obese nor diabetic.[8] The prevalence of NAFLD and NASH in the general population is estimated to be between 10%-24% and between 1%- 5%, respectively. There is a direct correlation between body mass index (BMI) and prevalence and severity of NAFLD. The prevalence of NAFLD increases to 57.5% to 74% in obese persons and 90% in morbidly obese persons.[4,7,9] The most common risk factors for NAFLD are obesity, type 2 diabetes mellitus, and hyperlipidemia.[4,7,9] Other common associations are hypertension, hyperuricemia, and polycystic ovary syndrome. The prevalence of obesity, type 2 diabetes, and hyperlipidemia in patients with NAFLD range from 30% to 100%, 10% to 75%, and 20% to 92%, respectively.[4,7,9] A plethora of case series of NAFLD have been reported over the past three years but whether this indicates a true increase in prevalence or simply an increased awareness of the disorder is unclear. However, it seems to be a true rise in prevalence of NAFLD in view of the present epidemic of obesity that also includes developing countries. The recent studies have shown that obesity is increasing in both children and adolescents

Table 1: Conditions associated with NAFLD

1. **Nutritional**
   a. Obesity
   b. Prolonged starvation
   c. Total parenteral nutrition
   d. Rapid weight loss
2. **Metabolic**
   a. Diabetes mellitus
   b. Hypertriglyceridemia
   c. Dysbetalipoproteinemia
   d. Limb lipodystrophy
3. **Drugs**
   a. Synthetic estrogens
   b. Corticosteroids
   c. Amiodarone
   d. Bleomycin
   e. Tetracycline
   f. Methotrexate
   g. Perhexiline
   h. Diltiazem, nifedipine
   i. Tamoxiphen
4. **Chemicals**
   a. Hydrocarbons
   b. Yellow phosphorus
5. **Surgery**
   a. Jejunoileal bypass
   b. Gastropexy
   c. Extensive small bowel loss

suggest that the prevalence of NASH is likely to go on increasing in the future.[10]

With the improvement in economic conditions there has been a change in lifestyle in India especially in the urban regions. Busy routines, job pressures have affected the dietary habits and there is hardly any time for exercise. Epidemiological data indicate the increasing incidence of obesity and diabetes mellitus in India.[11] Prevalence of diabetes mellitus is around 13.9% with a higher prevalence in urban population than in the rural population.[12,13] The percentage of patients taking anti-lipid drugs has also gone up. All these factors could lead on to insulin resistance and its variable manifestations including NAFLD. While 2% of patients attending liver clinic at our institute were found to be suffering from NAFLD, the prevalence of fatty liver on ultrasonography was 5%.[14] NAFLD also constitutes as the third most common cause of chronic liver disease in our clinical practice following alcoholic and virus-related liver disease (unpublished observation).

ETIOPATHOGENESIS

The pathogenesis of the NASH is not completely understood. Various conditions associated with NAFLD include nutritional abnormalities, metabolic disorders, drugs, chemicals and surgery (Table 1).[4,7,9,15] All these conditions initially lead on to steatosis which later, after a cascade of events, may progress on to have associated inflammation and fibrosis in some patients (NASH with fibrosis) and finally to cirrhosis.[15,16]

For the purpose of mechanisms leading to inflammation, fibrosis and cirrhosis from a relatively benign stage of steatosis, NASH may be considered at least a two hit process, i.e. the accumulation

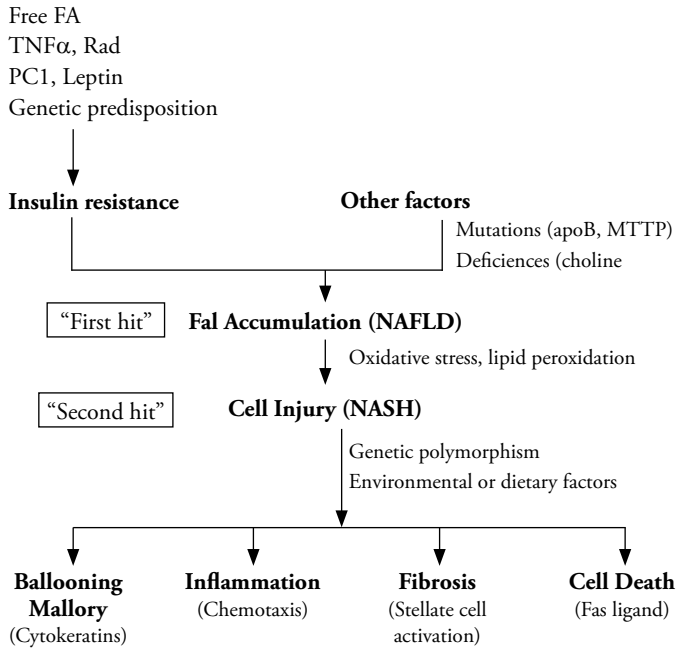

Fig. 2 : Mechanism of NASH

of the fat as the first hit and the hepatocellular injury in the fatty liver as the second hit (Figure 2).[16] The first hit of steatosis occurs because of the imbalance between the fatty acid uptake, its oxidation, esterification and export as very low-density lipoprotein (VLDL) from the hepatocytes. Insulin resistance is thought to be the key factor,[4,9,17,18] which leads to increase in lipolysis and increased uptake of fatty acids by hepatocytes. Hyperinsulinemia occurring as a result of insulin resistance also increases the intrahepatocytic fatty acids by increasing the glycolysis and decreasing the apolipoprotein B-100 and resulting in decreased export as VLDL. The end result is the increase in fatty acids and triglycerides in the hepatocytes leading to steatosis. The increased load of fatty acids in the hepatocytes increases the mitochondrial β-oxidation and increase in cytochrome P-450 4A and cytochrome P450 2E1 levels, leading to increase in reactive oxygen species. The increased mitochondrial oxidative stress leads to the second hit from steatosis to steatohepatitis and fibrosis by three main mechanisms, namely (i) lipid peroxidation, (ii) cytokine induction, and (iii) Fas ligand induction.[4,9,16]

i. Lipid peroxidation causes oxidative destruction of polyunsaturated fatty acids of cellular membranes. The cytotoxic products released due to lipid peroxidation may also impair cellular functions including nucleotide and protein synthesis leading to cell death, formation of Mallory hyaline, promoting tissue inflammation, activation of stellate cells and collagen synthesis.[9]
ii. Cytokines like IL-1, IL-6 IL-8 and tumor necrosis factor-α (TNF-α) plays an important role in the pathogenesis of liver injury in patients with NASH.[17] IL-1, IL-6, and IL-8 are pro-inflammatory cytokines and IL-10 and IL-12 are anti-inflammatory cytokines. TNF-α, TGF-β, interleukin-8 and cause chemotaxis, formation of Mallory hyaline and synthesis of collagen by activated stellate cells. TNF–α

Table 2: Clinical features and Investigations

1. **Symptoms and physical findings**
   Asymptomatic (30%-40%), right upper subcostal complaints (pain or discomfort, ~30%-40%), fatigue (<10%), edema (legs), gastrointestinal bleeding (if portal hypertension develops), abdominal distension (ascites, if portal hypertension or cirrhosis develops)
2. **Clinical signs**
   Normal examination, increased body mass index (BMI), increased waist circumference (central obesity), stigmata of chronic disease (if cirrhosis is present), lipomatosis, lipoatrophy or lipodystrophy, hepatomegaly (50%-70%), edema, spleen enlargement, and ascites
3. **Investigations**
   a. **Biochemical**
      i. Raised aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - up to 4-5 times elevation
      ii. Alkaline phosphatase - up to 2 times elevation
      iii. AST/ALT - majority <1; >1 may indicate cirrhosis
      iv. Bilirubin - elevated in late stage
      v. Albumin - decreased in late stage
      vi. Prothrombin time - prolonged in late stage
      vii. Serum markers of iron overload - ~25% but do not indicate hemochromatosis
      viii. Anti-nuclear antibody - ~ one-third
   b. **Imaging**
      i. Ultrasound - fatty liver
      ii. Computed tomography
      iii. Magnetic resonance imaging

downregulates insulin-induced phosphorylation of insulin receptor substrate 1 and reduces the expression of the insulin-dependent glucose transport molecule Glut 4 and thus contributes towards insulin resistance which is thought to be the major mechanism in the pathogenesis of NASH. Source of TNF- α include either the intestinal bacteria through the release of ethanol or lipo-polysaccharides or through IKKB activation through oxidative stress.[17]

iii. Finally the expression of Fas ligand due to oxidative stress leads to fractional killing by interaction with Fas on other hepatocytes.[9]

Other than insulin resistance other factors involved in the pathogenesis of NASH include serum and liver iron,[19,20] leptin,[21] bacterial overgrowth[22] and TNF-α.[23,24] Saturation of mitochondrial β-oxidation leads to peroxisomal oxidation and generation of hydrogen peroxide, which in the presence of increased iron is converted to hydroxyl radicals, thus adding to the oxidative stress and further injury.[25] We studied the serum and liver iron parameters in 48 of our patients with NASH. Only two patients were found to have abnormal serum iron ferritin and four patients were found to have abnormal transferrin saturation. In addition, HFE gene mutations (C282Y and H63D) were also studied in 28 of these patients. Only four patients were found to be heterozygous for H63D gene and none of the patients had C282Y gene mutation.[20]

Leptin, a product of obesity gene regulates the food intake and body composition through a central feed back mechanism and is proposed to be a key pathophysiological factor for obesity. Leptin leads to hepatic steatosis by promoting insulin resistance or by modulating insulin signaling in hepatocytes.[21] Bacterial overgrowth in the gut can cause liver injury by causing endotoxemia and release of various cytokines. The mechanisms by which intestinal bacteria may increase hepatic oxidative stress include increased endogenous production of ethanol and by direct activation of inflammatory cytokines in luminal epithelial cells, and liver macrophages or both via release of lipopolysaccharide.[22]

TNF-α is derived from adipose tissue in the absence of active infections or inflammatory conditions. Plasma levels of TNF-α also correlates with body fat mass and is associated with insulin resistance.[23] TNF-α knockout mouse also fails to develop insulin resistance after induction of obesity suggests its crucial role in the pathogenesis of insulin resistance.[24] The oxidative stress and cytokine production leads to the IkKβ activation which in turn causes further release of TNF- α and insulin resistance though the activation of NF-kβ and the cycle goes on, which ultimately leads to steatohepatitis.[9]

NASH AS A MANIFESTATION OF INSULIN RESISTANCE SYNDROME (METABOLIC SYNDROME)

The evidence is increasing that NAFLD and NASH represent the hepatic component of the metabolic syndrome of insulin resistance.[26,28] Metabolic syndrome is characterized by the presence of insulin resistance (hyperinsulinemia and peripheral insulin resistance) in association with other metabolic abnormalities like obesity, diabetes, hypertriglyceridemia and hypertension, etc. Common associations of NAFLD, such as, hypertension, hyperuricemia, and polycystic ovary syndrome, are also common to metabolic syndrome. Though NASH can occur in individuals with normal body weight and even in lean and thin individuals, as highlighted earlier, the chances of individual having NAFLD and NASH increase with increasing body weight, with 70-80% of obese individuals having NAFLD and 15-20% having NASH.[4,9] Conversely 30-100% of NASH patients have obesity. Truncal or central obesity, which is more strongly associated with NASH, also predisposes to diabetes and hypertension. In severely obese patients, the risk of liver disease progressively increases with the number of features of metabolic syndrome.[29,30] One of the recent studies which applied adult treatment panel III (ATP III) criteria for metabolic syndrome found 88 % of patients of NASH having metabolic syndrome.[27] However, metabolic syndrome was present in 22% of Indian patients with NASH .[31]

The prevalence of the metabolic syndrome is high among obese children and adolescents, and it increases with worsening obesity.[10]

CLINICAL FEATURES AND DIAGNOSIS OF NAFLD

Currently there is no noninvasive test to diagnose NAFLD; liver biopsy remains the most sensitive test but cannot distinguish NAFLD from other causes of fatty liver disease (Table 1). By definition NAFLD is a chronic liver disease most patients are asymptomatic in the beginning with incidental detection of raised liver enzymes (Table 2). In fact NASH is now considered

Table 3: Diagnosis of NASH

1.	**Presumptive diagnosis**
	a. Elevated serum liver enzyme levels [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]
	b. Imaging showing the evidence of fatty liver
	c. Insignificant alcohol intake
	d. Absence of hepatotoxic drugs intake
	e. Negative test results for viral hepatitis
	f. Negative test results for autoimmune liver disease (autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis)
	g. Negative test results for metabolic disorder, such as, Wilson's disease and hemochromatosis
2.	**Definitive diagnosis**
	Liver biopsy specimen shows evidence of fatty change (macrovesicular > microvesicular) accentuated in zone 3, with lobular inflammation and hepatocellular ballooning. Pericellular fibrosis in zone 3, hepatocellular glycogenated nuclei, lipogranulomas and occasional acidophil bodies may also be present.

the most important cause of unexplained asymptomatic rise in transaminases in a nonalcoholic individual.[6] Many of patients with NASH may complain of fatigue and mild pain in the right upper quadrant of abdomen, which may sometimes be mistaken for gallstone disease (Table 2). Mild hepatomegaly may be an important sign but signs of liver failure are absent unless the patient has developed cirrhosis liver. Serum biochemistry usually shows mildly elevated AST and ALT (Table 2). Other liver functions are usually preserved. Imaging (ultrasonography, computed tomography and magnetic resonance imaging) shows changes of fatty liver disease and the diagnosis is clinched by the liver biopsy. The criteria by Powell et al[32] originally described for the diagnosis of NASH included a liver biopsy showing steatohepatitis, convincing evidence of minimal or no alcohol consumption (< 40 g/wk) and exclusion of viral infections. Though there are no universally accepted limits for alcohol intake, but an alcohol intake of < 20 g/d is unlikely to cause the fatty liver, and is usually taken as a defining criteria for NASH.[4] Because there are no specific tests for NASH, the diagnosis remains one that is established after the exclusion of other causes of chronic liver disease (Table 3).

NASH and liver biopsy

NASH by definition is a histological diagnosis. Typically histological features of NASH are the presence of macrovesicular steatosis, lobular neutrophilic inflammation with additional presence of Mallory bodies, ballooning degeneration, lipogranuloma and pericellular fibrosis.[33] Such liver damage predominates in perivenular regions, i.e., zone 3 of hepatic acinus. The liver damage may lead to fibrogenic response that sometimes progress from pericellular fibrosis to bridging fibrosis and ultimately to cirrhosis. Since NAFLD is thought to have a benign long-term course, doing a liver biopsy in these patients is controversial.[34,35] Arguments in favour of biopsy include: (i) exclusion of alternative causes of liver disease, (ii) to distinguish steatosis from NASH, (iii) estimation of prognosis based on degree of fibrosis, and (iv) determination of progression of fibrosis over time. Arguments against biopsy include: (i) generally good prognosis of NAFLD, (ii) lack of effective therapy, and (iii) the risks and costs associated with biopsy. Predictors of severe liver fibrosis (bridging fibrosis/cirrhosis) on liver biopsy in patients with NAFLD include old age, presence of diabetes mellitus, obesity, AST:ALT more than 1, ALT ≥ 2 times normal and triglycerides ≥ 1.7 mmol/L.[30,36,37] This is the subgroup of patients with NASH who would be expected to derive the most benefit from having a liver biopsy and for considering therapy. We have also found increasing BMI and AST levels to be determinants of significant liver injury in patients with NASH.[38]

NASH and cryptogenic cirrhosis

Like any chronic liver disease NASH may be asymptomatic in the beginning and may present later as cryptogenic cirrhosis or even HCC. Since NASH is a recently recognized entity, some of the patients who had NASH in the past and remained unrecognized could now be coming with cirrhosis liver of unknown etiology. It may be difficult in these patients to recognize NASH even on histology because the characteristic changes of NASH may not be evident once it goes into the stage of cirrhosis. In a case control study Poonawala et al[39] found that patients with cryptogenic cirrhosis more commonly had obesity and presence of diabetes in comparison to patients with cirrhosis due to alcohol, viruses, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). To test the hypothesis that NASH may progress to cirrhosis and may be responsible for patients with cryptogenic chronic liver disease, Caldwell et al[40] compared the prevalence of risk factors like diabetes and obesity in patients with cryptogenic cirrhosis, NASH, PBC and HCV-related cirrhosis. They found that diabetes and obesity prevalence was more common in patients with cryptogenic cirrhosis and NASH compared to those with PBC or HCV-related cirrhosis. On these observations they suggested that NASH might be an unrecognized cause of cryptogenic cirrhosis.[40] In a recent Indian study NASH with or without cirrhosis, and cryptogenic cirrhosis occurred more commonly in patients with diabetes than those without it. The incidence of diabetes in cryptogenic cirrhosis was 57% versus 30% in non-cryptogenic cirrhosis.[41] Our data also confirms higher prevalence of increased BMI, low HDL and diabetes mellitus in patients with cryptogenic cirrhosis as compared to controls.[42]

Natural history of NASH

The natural history of NAFLD is not well defined. Recent reports suggest that 28% of patients have progression on histological examination, 59% have no change, and 13% have improvement.[4] Matteoni et al[43] retrospectively determined the histological and/or clinical outcome of 98 patients with the whole spectrum of NAFLD from simple steatosis through NASH to cirrhosis. After a median 8-year follow up, 25% of individuals with evidence of hepatocyte necrosis with or without Mallory's hyaline or fibrosis, either already had cirrhosis on index biopsy or progressed to cirrhosis. This compared with only 3.4% of patients with simple steatosis with or without non-specific inflammatory changes. These observations indicate that NAFLD patients without NASH have a benign prognosis.[43]

Table 4: Treatment of NASH

1. **Current therapeutic approach**
   a. Lifestyle modifications
      i. Weight loss
      ii. Exercise
2. **Treatment of NASH-related pathologies**
   a. Control of diabetes mellitus
   b. Lipid lowering agents
3. **Potential therapies**
   a. Insulin sensitizing agents
      i. Biguanides (metformin)
      ii. Thiazolidinediones (rosiglitazone, pioglitazone)
      iii. Leptin (not available commercially)
   b. Antioxidants
      i. Natural antioxidants (vitamin E, ubiquinone)
      ii. Synthetic antioxidants (including dihydroquinoline-type – only used experimentally)
      iii. Selenium
      iv. Betaine
      v. N-acetylcysteine
   c. Other
      i. Ursodeoxycholic acid
      ii. Metadoxine
      iii. Lecithin
      iv. Silymarin
4. **Liver transplantation** (only in end-stage liver disease)

TREATMENT

No therapy for NASH has clearly been proven effective; the pharmacological treatment of patients with NASH is still evolving. Therapeutic modalities have been applied according to the risk factors of NASH. Different treatment modalities for NASH include lifestyle modifications such as weight loss and exercise, treatment of NASH-related pathologies such as control of diabetes mellitus and control of hyperlipidemia, insulin sensitizing agents such as biguanides (metformin), thiazolidinediones (rosiglitazone, pioglitazone), antioxidants etc (Table 4). Dietary restriction, exercise and weight reduction, which improve insulin sensitivity, are usually the first-line of treatment. Weight reduction in obese has been found to improve the serum ALT levels and liver steatosis.[44,45] The weight reduction has to be slow and sustained with adequate intake of essential amino acids because rapid weight loss can rather worsen the necro-inflammation. The recommendations are no more than 1.6 kg of weight loss in a week with a maximum of 10% of the baseline over a period of six months. This weight reduction is achieved by the dietary fat restriction to < 20g/d and with moderate sustained exercise like swimming, jogging, cycling, running etc. Since diabetes mellitus and hyperlipidemia are other risk factors associated with NASH, control of blood sugars and lipids is also recommended. Various lipid-lowering drugs tried in NASH include, clofibrate,[46] gemfibrozil,[47] atorvastatin,[48] pitavastatin[49] and probucol.[50] Various antidiabetic agents mainly used to improve insulin sensitivity in patients with NASH are metformin, troglitazone, rosiglitazone and pioglitazone. Metformin by improving the insulin sensitivity and by its anti-TNF action has been shown it to be useful in both humans and animal models of NASH.[51,52] In our preliminary report, we have also shown to be useful in those not responding to the lifestyle modifications and UDCA.[18] Troglitazone was found to be useful in one of the studies but because of the hepatotoxicity has been banned by the FDA.[53] In recent studies both rosiglitazone and pioglitazone were found to be effective in improving the serum ALT and histology in patients with NASH.[54,55] Initial studies[46,56] found ursodeoxycholic acid to be an effective drug in improving the serum biochemistry in NASH patients but in a recent, one of the largest, placebo-controlled randomized trial, UDCA was found to be no better than the placebo in patients with NASH.[57] This study highlights the importance of having a control group while studying the efficacy of drugs in NASH.[57] Vitamin E has antioxidant properties and since oxidative stress plays an important role in the pathogenesis of NASH, it has a role in the treatment of patients with NASH. In one of earlier pilot studies Lavine et al[58] used vitamin E in children with NASH and found to be effective in improving the serum ALT levels. On the other hand, Kugelmas et al[59] while studying the role of cytokines, diet, exercise and vitamin E found that the results were not changed with the addition of vitamin E to the lifestyle modifications. Dose and duration of drug is important in studying the efficacy of vitamin E. Since cytokines mainly TNF is important in causing injury in patients with NASH, there is rationale in treating these patients with anti-TNF drugs. In a study on ob/ob mice with NASH anti-TNF antibodies were found to be effective in improving the histology and ALT levels.[60] Probiotics may also have role in the treatment of NASH because intestinal bacteria could be the source of TNF.[60,61]

MAIN POINTS

1. NAFLD encompasses a broad clinicopathologic spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) that may progress to cirrhosis and end-stage liver disease.
2. NASH is characterized by similar histopathological features to those observed in alcoholic liver disease, but occurs in the absence of significant alcohol consumption.
3. The prevalence of NAFLD and NASH in the general population is estimated to be between 10%-24% and 1%-5%, respectively.
4. The most common risk factors for NAFLD are obesity, type 2 diabetes mellitus, and hyperlipidemia.
5. The recent studies indicate that obesity is increasing in both children and adolescents, thereby suggesting that the prevalence of NAFLD is likely to go on increasing in the future.
6. The pathogenesis of NAFLD consists of hepatic fat accumulation because of insulin resistance and hepatocellular injury in the steatotic liver because of oxidative stress with formation of free radicals, etc.
7. The presumptive diagnosis of NASH is based on elevated serum liver enzyme levels and exclusion of alcohol abuse as well as viral, autoimmune, genetic, and drug-induced liver diseases. Liver biopsy is essential for definitive diagnosis but may not be necessary for clinical management.
8. Treatment is aimed at correcting the risk factors for NAFLD, such as, medical control of hyperglycemia, and use of lipid-lowering agents for hypertriglyceridemia, and lifestyle

modification and pharmacological agents to improve insulin sensitivity.

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