Non-alcoholic fatty liver disease: an under-recognized cause with emerging importance

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Non-alcoholic fatty liver disease (NAFLD) and its more aggressive form, non-alcoholic steatohepatitis (NASH) are entities that are becoming more and more interesting to the medical community in general. The increased prevalence of diabetes, obesity, hypertension and hypertriglyceridemia are considered to be important causes for NAFLD. The prognosis of simple NAFLD is generally benign. But fibrosis, ballooning of the hepatocytes, inflammation and Mallory bodies are indicators of progression to cirrhosis. Although liver biopsy is currently the gold standard for diagnosis, there is a need for developing less invasive methods, and hence there is no effective medical therapy available for NAFLD. A better understanding of the pathogenesis and natural history of NASH will help identify the subset of patients at risk of progressing to advanced liver disease.

Keywords: Insulin resistance, non-alcoholic fatty liver disease, obesity, steatohepatitis.

NON-alcoholic steatohepatitis (NASH) is a distinct hepatic disorder observed in patients without a history of significant alcohol consumption, that histologically resembles alcohol-induced liver damage. It was first described in obese and diabetic women[1]. NASH is considered to be part of the spectrum of non-alcoholic fatty liver disorders (NAFLD), ranging from bland steatosis to steatohepatitis and cirrhosis[2–4]. NAFLD has four histological stages: (i) Fatty infiltration of the liver; (ii) Fatty infiltration plus inflammation; (iii) Fatty infiltration with ballooning degeneration; (iv) Fatty infiltration with lesions similar to alcoholic hepatitis and sinusoidal fibrosis, polymorphonuclear infiltration with or without Mallory hyaline. NASH is the name given to the third and fourth stages[4].

NASH has been associated with insulin resistance, which includes obesity, diabetes, hypertriglyceridemia and hypertension[5,6] (Table 1)[7–11]. In addition, NASH has also been associated with hyperlipoproteinemia, jejunal bypass[12,13], parenteral nutrition, drugs (tamoxifen, steroid, 'massive' estrogen, amiodarone, antiviral drugs – nucleoside analogues – aspirin/NSAIDS, methotrexate, nifedipine, perhexiline maleate, tetracycline, valproic acid)[14], α_1-anti-trypsin deficiency[15], bacterial overgrowth[16] and environmental toxins[14,17,19]. Cotrim *et al.*[20] suspected exposure to chemicals (benzene, xylene, ethylene, dimethylformamide, vinyl chloride and others) is another risk factor. There is an increasing body of evidence that some cases of cryptogenic cirrhosis[21], requiring liver transplantation could have been the result of NASH[22]. Iron overload, H63D mutation of the *HFE* gene[23], and immune system anomalies are frequent in patients with non-alcoholic steatohepatitis[24]. Recent reports have also described hepatocellular carcinoma arising in patients with NASH-associated cirrhosis[25].

Paediatric NAFLD

Reports of NAFLD[26] in children first appeared in the early 1980s. Since then a number of case series of childhood NAFLD have been reported, including cirrhosis[10,11,27]. Contributing factors are controversial, but include obesity, cranial irradiation, drugs (such as corticosteriods, L-asparaginase), and the presence of protein-calorie malnutrition[28–30].

A number of childhood conditions are associated with hepatic steatosis. They are mainly of two groups: First, in syndromes or conditions associated with obesity where insulin resistance may be present, or where obesity is a secondary phenomenon. Examples include Bardet–Biedl syndrome, Alstrom syndrome and Turner syndrome[31]. Secondly, in lipodystrophy/lipoatrophy syndrome, where insulin signalling is defective resulting in insulin resistance[32].

Epidemiology

With emerging urbanization, increasing affluence and behavioural changes of physical inactivity and high fat/energy-excessive diet, type-2 diabetes has become common in Asia and the western Pacific rim. The true prevalence of NAFLD among the various racial and ethnic subgroups is not fully characterized. The rates range from 7 to 40%, which in countries like Japan represents a 3 to 20-fold increase over the last 20 years. The increase is associated with central adiposity, insulin resistance, hepatic steatosis

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Table 1. Different studies showing factors associated with NASH

Study	*N*	Age (yrs)	Female (yrs)	DM (%)	Obese (%)	Hyperlipidaemia (%)
Ludwig *et al.*[1]	20	54	65	25	90	67
Powell *et al.*[7]	42	49	83	36	93	81
Bacon *et al.*[8]	33	47	42	21	39	21
Matteoni *et al.*[4]	132	53	53	33	70	92
Angulo *et al.*[9]	144	51	67	28	60	27
Baldridge *et al.*[10,*]	12	14	33	0	100	83
Rashid and Roberts[11,*]	36	12	42	11	83	31

*Paediatric studies.

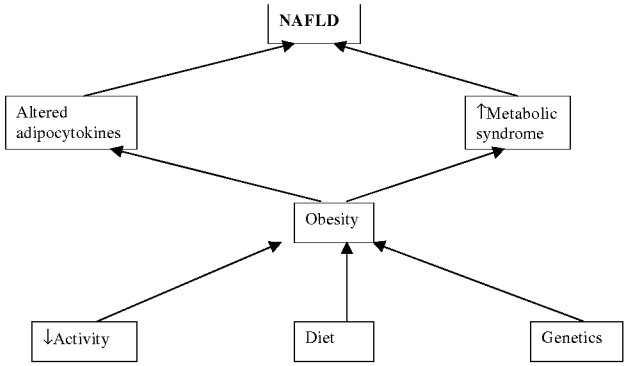

Figure 1. Schematic diagram showing relationship between fatty liver disease and metabolic syndrome.

and NASH. After cancer, cirrhosis from NASH is now the secondmost common age-related cause of death in type-2 diabetes. Emphasis will be on lifestyle adjustments (physical activity and diet) to prevent or reverse fatty liver disorders[33]. The prevalence of NAFLD is about 10–24% in common population, and 57.5% among people with obesity. It was reported[34] in Japan that the incidence of NAFLD in children with obesity is around 22.5–52.8%.

Pathogenesis

The pathogenesis of NASH is unknown. In 1998, Day and James[35] first proposed the 'two hit' hypothesis for pathogenesis of NASH. Fatty liver, the earliest and most prevalent stage of NAFLD[36], is thought to sensitize the liver to additional necroinflammatory insults[35], thus promoting disease progression to steatohepatitis, cirrhosis and hepatic failure[1,4,37]. A number of factors point to the multifactorial nature of this disease, including derangement in metabolic parameters, endotoxin-induced cytokine release and oxidative stress[35,38] (Figure 1).

After absorption from the intestines, fat is carried to the adipose tissue for storage in the form of triglycerides. It is released as free fatty acids (FFA) when the body is deprived of food or under the effect of certain hormones/drugs (such as epinephrine, corticosteroids). FFA are carried to the liver bound to albumin. After entering the hepatocytes they are either oxidized to produce energy or resynthesized and transported back to the adipose tissue bound to very low-density lipoproteins (VLDL). Fatty acids are also synthesized by hepatocytes when there is dietary excess of carbohydrates. Accumulation of fat in the liver can occur because of: (i) increased delivery of FFA to the liver, (ii) increased synthesis of fatty acids in the liver, (iii) decreased β-oxidation of FFA, and (iv) decreased synthesis or secretion of VLDL[39,40]. The two main pathways of hepatocellular injury are considered to be oxidative stress-induced lipid peroxidation and cytokine-mediated injury.

Cytokines and NASH

Cytokines are attractive candidates for the 'second hit' in the pathogenesis of NASH. They are capable of producing all the classical histological features of NASH, including hepatocyte death/apoptosis (TNF-α), neutrophil chemotaxis (IL-8) and hepatic stellate cell activation (TNF-α, TGF-β)[41]. There is evidence that endotoxin-mediated cytokine release is important in the occurrence of hepatic steatohepatitis[42], and that the use of antimicrobial therapy may be able to prevent or reverse its development. In addition, it has been shown that patients with NASH had an increased expression of TNF-α mRNA both in their liver and adipose tissue compared to obese controls, and this over-expression correlated with histological severity[43].

Oxidative stress and lipid peroxidation

There is growing evidence implicating FFA in the production of oxidative stress within hepatocytes. Increased fatty acid β-oxidation as well as peroxisomal fatty acid oxidation can both lead to increase in reactive oxygen species generation and subsequent lipid peroxidation. In the fasting state, patients with NAFLD have increased plasma levels of β-OH butyrate[44].

Under normal conditions, hepatic aerobic metabolism involves a steady-state production of pro-oxidants such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are balanced by a similar rate of their consumption by antioxidants[45]. Imbalance in the pro-oxidant/antioxidant equilibrium in favour of pro-oxidants consti-

tutes the oxidative stress phenomenon, a condition that may induce a number of pathophysiological events in the liver[45–47]. Hepatotoxicity by oxidative stress may be achieved through a direct attack of ROS and RNS on essential biomolecules with loss of their biological functions and cell viability[45–47]. Alternatively, ROS may indirectly activate redox sensitive transcription factors such as nuclear factor κB (NF-κB)[48] or activator protein-1 (AP-1)[49], thus triggering the production of cytotoxic, proinflammatory and/or fibrogenic mediators by Kupffer cells and other non-parenchymal cells[50]. These studies suggest that chronic oxidative stress may be important in the progression of NAFLD.

Pessayre *et al.*[41] have shown that excess fat deposition in the liver is associated with lipid peroxidation and the degree of this peroxidation is directly related to the severity of steatosis. The end-products of lipid peroxidation, 4-hydroxynoneal and malondialdehyde, covalently bind to hepatic proteins, and act as potent agents for neutrophil chemotaxis and stimulating pro-inflammatory cytokines. Malondialdehyde also activates hepatic stellate cells to produce collagen, leading to fibrosis.

Insulin resistance

The association between the severity of insulin resistance/presence of NIDDM, and the risk of NASH can be explained by peripheral insulin resistance increasing the supply of FFA to the liver and by hepatic insulin resistance favouring the development of oxidative stress. The increase in supply of FFA to the liver leads not only to steatosis, but may also contribute to the hepatic insulin resistance observed in humans with NAFLD, who have demonstrated impaired insulin-mediated suppression of hepatic glucose production compared with controls[44,51].

Other factors

In addition to obesity and insulin resistance, some other environmental or genetic factor(s) is required for the progression of NASH. Studies in leptin-deficient ob/ob mice which have profound insulin resistance and dramatic hepatic steatosis without steatohepatitis or fibrosis, suggests that leptin may in fact have a role in promoting hepatic fibrogenesis, directly by an autocrine effect on hepatic stellate cells and indirectly by up-regulating the production of TGF-β from sinusoidal endothelial cells and Kupffer cells[52].

The association of hepatic iron accumulation and NAFLD continues to be debated. While some studies have found that 22 to 62% of individuals with fatty liver disease have evidence of iron overload[8], other have failed to show such relationship[9,53]. In another study, a higher incidence of the HFE mutation (Cys282Tyr) was reported[21].

Candidate genes

NASH and cryptogenic cirrhosis study suggest that genes might play an important role in NAFLD[21]. Day[54] identified different types of candidate genes of NAFLD as follows: genetic factors related to insulin resistance, FFA supply and lipid metabolism. Apolipoprotein E, a regulator of lipoprotein metabolism, was included and considered to be of great importance. Genes associated with the 'second hit', include (i) genes encoding proteins involved in the severity of oxidative stress such as HFE (haemochromatosis gene), CYP2E1, CYP4A; (ii) genes encoding cytokines and their receptors; (iii) genes related to adverse effects of FFA such as transcription factors, peroxisome proliferator-activated receptors (PPARs). Among these candidate genes are: (a) leptin and its receptor, which are related to obesity, insulin resistance, increased FFA synthesis and reduced FFA oxidation; (b) PPAR regulating a variety of genes encoding enzymes involved in FFA oxidation and oxidative stress; and (c) PPAR which up-regulates UCP2 (un-coupling protein C) and inhibits leptin gene expression and macrophage function. Up-regulation of UCP2 leads to reduction of ATP.

Diagnosis

Most patients with NAFLD are asymptomatic[7,8,55–57] with moderately elevated aminotransferase levels, particularly ALT[58]. The vast majority of individuals with NAFLD are diagnosed incidentally during the course of assessment of unrelated symptoms or the associated metabolic syndrome. Differential diagnosis between simple steatosis and steatohepatitis is of vital importance because the former has a benign process, while the latter tends to develop into advanced fibrosis or even cirrhosis. Conventional liver tests cannot differentiate between alcoholic and non-alcoholic hepatitis[58–60]. On physical examination, obesity is often the dominating finding. Elevated ALT levels observed in NASH, which appear to be a consequence of steatosis, might involve balloonization necrosis. γ-glutamyl transpeptidase and alkaline phosphatase can be mildly elevated, but bilirubin, albumin and prothrombin time are usually normal unless the disease is advanced. The AST/ALT ratio is however greater than 1 in alcoholic liver disease and less than 1 in non-alcoholic steatohepatitis[61]. Immunoserologic findings compatible with autoimmune hepatitis are commonly present with primary NASH[62,63].

Diagnosis of primary NASH must include a negative evaluation for chronic hepatitis C virus infection (antibody to hepatitis C virus) and hepatitis B virus infection (hepatitis B surface antigen). Ceruloplasmin levels, α-1-anti-trypsin levels are usually normal in patients with NASH. Idiopathic genetic hemochromatosis must be excluded even in the presence of elevated levels of serum ferritin and transferin saturation. Autoimmune serology

(antimitochondrial antibody, antinuclear antibody, antismooth muscle antibody, and anti-liver/kidney microsomal antibody) should remain negative in patients with NASH, except for some patients presenting with low titre antinuclear antibody positivity[56].

Diagnosis of NAFLD is based on two criteria: (i) establishing the presence of a fatty liver or steatohepatitis, and (ii) establishing the nonalcoholic nature of the disease process. Radiologic imaging of the liver with sonography, computed tomography (CT), or magnetic resonance imaging (MRI) has an adequate threshold for detection of fatty infiltration of the liver, used either singly or in combination. Each of these modalities has its own pitfalls and cannot distinguish steatosis from steatohepatitis. These methods are also insensitive in detecting steatosis of less than 25 50 30%[64].

Liver biopsy is the gold standard for diagnosis of NAFLD/NASH for the following important reasons: (i) to confirm diagnosis and establish severity of fibrosis and presence of cirrhosis, and (ii) to exclude other coexisting conditions that can result in hepatitic steatosis. However, ethical consideration as well as inherent risk associated with this procedure limit its widespread applicability.

Histological diagnosis of steatohepatitis relies on a constellation of lesions that include steatosis (mainly macrosteatosis, occasionally microsteatosis), ballooning of hepatocytes (hepatocyte injury), perisinusoidal fibrosis and a mixed lobular inflammatory infiltrate[65]. Currently, minimal histological criteria required for diagnosis are the presence of steatosis and intralobular necrotic inflammatory reactions[59]. Focal necrosis is usually centrilobular[59] and the cellular response involves lymphocytes, mononuclear cells and neutrophils. The ultrastructure of Mallory bodies in patients with NASH is similar to that seen in patients with alcoholic hepatitis[60]. Mallory bodies are not now considered necessary for diagnosis[23]. There is not, however, general agreement on one single histological description[3]. Diagnosis of NASH thus requires careful examination of both the clinical signs and anatomic findings. Different grading and stages of histological variables are recommended for NASH analysis (Table 2)[66].

Table 2. Grading and stages of NAFLD[66]

Grade of NAFLD
Macrovesicular steatosis
Grade 0: No steatosis
Grade 1: < 33% steatosis
Grade 2: < 33–66% steatosis
Grade 3: > 66% steatosis
Necroinflammatory activity
Grade 1 (mild) steatosis up to 66%; occasional ballooned hepatocyte (mainly zone 3); scattered intra-acinar neutrophil (PMN) lymphocytes, no or mild portal inflammation.
Grade 2 (moderate) steatosis of any degree; obvious zone-3 ballooning degeneration; intra-acinar PMNs; zone-3 perisinusoidal fibrosis may present mild to moderate, portal and intra-acinar inflammation.
Grade 3 (severe) panacinar steatosis; widespread ballooning; intra-acinar inflammation; PMNs associated with ballooned hepatocyts, mild to moderate portal inflammation.
Stage of NAFLD
Stage 1: zone 3 perisinusoidal/pericellular fibrosis; focally or extensively present.
Stage 2: zone 3 perisinusoidal/pericellular fibrosis with focal or extensively periportal fibrosis.
Stage 3: zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis with focal or extensive bridging fibrosis.
Stage 4: cirrhosis.

Treatment strategies

Currently, there are no effective therapies for NASH, as its natural history and prognosis are not well understood. Treatment of patients with non-alcoholic fatty liver has typically been focused on the management of associated conditions such as obesity, diabetes mellitus, and hyperlipidemia as well as discontinuation of potentially hepatotoxic drugs. Appropriate metabolic control for patients with diabetes mellitus or hyperlipidemia is recommended, but is not always effective in reversing non-alcoholic fatty liver.

Non-alcoholic fatty liver associated with obesity may resolve with weight reduction[67–69], although the benefits of weight loss have been inconsistent. On the other hand, striking weight losses have also been associated with progression of the disease[70]. Moderate and gradual weight loss can safely improve in chronic liver disease associated with obesity and diabetes[71]. Rapid weight loss may aggravate the histologic lesions of steatohepatitis[70]. A weight loss of 500 g per week in children and 1600 g per week in adults is recommended, although the most appropriate rate of weight loss is still to be established.

Gastric bypass or gastroplasty performed in obese patients has significantly reduced steatosis[72]. Discrete inflammatory changes also largely disappeared and serum alkaline phosphatase was significantly reduced[73]. In contrast, weight loss induced by jejunoileal (J–I) bypass operations, in most cases, seems to be accompanied by a further increase in liver steatosis and fibrosis[74]. After jejunoileal bypass, steatohepatitis has been resolved with metronidazole therapy[42], but this treatment has not been evaluated in primary NASH.

A number of pharmacologic agents have been shown to be promising in the treatment of NASH (Table 3)[75–81]. Promising results of pilot studies evaluating ursodeoxycholic acid, gemfibrozil, betaine, *N*-acetylcysteine and alpha-tocopherol suggest that these medications may be of potential benefit in the treatment of patients with non-alcoholic fatty liver, but need further study in controlled trials[84]. In fact, a trial utilizing troglitazone had shown encouraging results[85]; but because of reports of rare but serious hepatotoxicity, the drug has now been withdrawn

Table 3. Pharmacologic treatment of NASH

Study	Drug	*N*	Type of study	Duration (months)	Compared with	AST/ALT	Histology
Laurin *et al.*[75]	Clofibrate	16	OL	12	Baseline	NC	NC
Basaranoglu *et al.*[76]	Gemfibrozil	46	RCT	1	Baseline	Improved	ND
Laurin *et al.*[75]	UDCA	24	OL	12	Baseline	Improved	Improved
Guma *et al.*[77]	UDCA + diet	24	RCT	6	Baseline Diet alone	Improved	ND
Abdelmalek *et al.*[78]	Betaine	8	OL	12	Baseline	Improved	Improved
Gulbahar *et al.*[79]	NAC	11	OL	12	Baseline	Improved	ND
Lavine *et al.*[80]	Vit E	11*	OL	4–10	Baseline	Improved	ND
Hasegawa *et al.*[81]	Vit E	22	OL	12	Baseline Diet	Improved	Improved
Caldwell *et al.*[82]	Troglitazone	10	OL	3–6	Baseline	Improved	Improved
Marchesini *et al.*[83]	Metformin	14	OL	4	Baseline	Improved	ND

*Study in children.
NAC, *N*-acetylcysteine; NC, No change; ND, Not done; OL, Open label; RCT, Randomized control trial; UDCA, Ursodeoxycholic acid.

from the market. The association of hyperinsulinemic insulin resistance has provided a target for treatment. Metformin, a biguanide that reduces hyperinsulinemia and improves hepatic insulin resistance has been shown to greatly reduce hepatomegaly and steatosis in mice and may potentially be useful in the treatment of NASH in humans[86].

Conclusion

NAFLD was once considered to be a relatively uncommon and benign condition restricted largely to middle-aged, obese, diabetic people. However, it has recently been recognized that NASH may be a relatively common liver disease occurring in individuals who are neither obese nor diabetic. Like non insulin-dependent diabetes mellitus and coronary heart disease, NASH may be considered a 'disease of affluence' and as a result is almost certainly on the rise. Of great concern are reports that NASH may be a progressive condition accounting for many cases of cirrhosis, previously considered to be 'cryptogenic' (no known cause). Understanding the pathogenesis of NASH is of great importance in ultimately finding a treatment, cure or means of prevention of this disease.

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