



Nonalcoholic fatty liver disease: Diagnosis, pathogenesis, and management

LIVER

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is an umbrella term that covers both a relatively benign condition, which is simple steatosis, and nonalcoholic steatohepatitis (NASH). NASH is characterized by a chronic and progressive liver pathology that may progress to cirrhosis, end-stage liver disease, hepatocellular carcinoma, and liver transplantation. Despite the growing body of evidence, one of the important and unresolved problems is the pathogenesis of NASH. It might be a metabolic disturbance as a primary abnormality in NAFLD. Insulin resistance is at the center of these metabolic abnormalities. Then, hepatocyte injury might be induced by oxidative stress. This ongoing process progresses to NASH, even to cirrhosis in some patients. In addition to oxidative stress, possibilities for the next hit are lipid peroxidation, reactive metabolites, adipose tissue products, transforming growth factor- β , Fas ligand, mitochondrial dysfunction, respiratory chain deficiency, and intestinal microbiota. Currently, there is no well-established and approved therapy. Recommendations are to improve existing co-morbidities, such as obesity, hyperlipidemia, or type 2 diabetes, and lifestyle modification with weight loss and exercise.

Keywords: Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, obesity, intestinal microbiota

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) defines a spectrum of histological abnormalities, from simple fatty liver to nonalcoholic steatohepatitis (NASH), in a person consuming no alcohol (1-6) (Table 1). NAFLD is a part of the metabolic syndrome, particularly in obesity, hyperlipidemia, and diabetes. The prevalence of NAFLD continues to increase, which depends on the increasing obesity prevalence due to economic development. NASH has the potential to progress to cirrhosis, hepatocellular carcinoma (HCC), end-stage liver disease, and liver transplantation. One of the most important and unresolved problems is the pathogenesis and underlying mechanisms in the development of NASH. The natural history of NAFLD is also not fully understood. The progression of NAFLD might be slower. However, the increasing prevalence of obesity as an epidemic according to World Health Organization (WHO) records

increases the importance of NAFLD burden and health budget burdens throughout the world.

The aim of this paper was to provide an overview of the current knowledge about NAFLD in light of new evidence. The therapy of NAFLD is widely discussed.

NAFLD: DEFINITIONS, PREVALENCE, AND RELATED FACTORS

Nonalcoholic fatty liver disease is the presence of excessive fat in the liver, demonstrated by imaging modalities, such as transabdominal ultrasound, computerized tomography, and magnetic resonance imaging (3-5). It is also a diagnosis of exclusion. Thus, patients must not have any other liver disease, such as hepatitis B and C, drug toxicity, autoimmune diseases, alcoholic liver disease, metabolic liver diseases, and genetic liver abnormalities. Patients must be abstinent from alcohol or drink infrequently.

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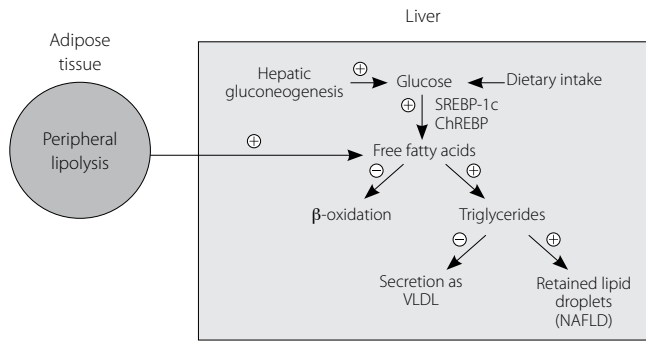


Figure 1. Pathogenesis of NAFLD and NASH.

The prevalence of NAFLD and NASH is 10% to 40% and about 2% to 5% in the American general adult population, respectively (6), and 20% and 1.2%-4.8% in other developed countries. Hispanics may progress to cirrhosis more frequently than either blacks or whites (7-9). Historically, an autopsy study showed a prevalence of NASH of 18.5% in markedly obese and 2.7% in lean subjects (10). Studies performed in general populations also showed that the prevalence of NASH is 3% in non-obese and 20% in obese subjects (11,12).

The prevalence of NASH-related cirrhosis and HCC is also high among patients with diabetes, as in obesity (13-16). As a risk factor, hypertriglyceridemia is also associated with insulin resistance and NAFLD, even in patients without obesity (3,12).

CLINICAL AND LABORATORY FINDINGS IN PATIENTS WITH NAFLD

Nonalcoholic fatty liver disease occurs equally in both genders (12). It is generally found within the fourth or fifth decade of life, even in children with high prevalence. Patients with NAFLD may have hypertension, besides obesity, type 2 diabetes, and hypertriglyceridemia (12-16). Many patients have no symptoms. The most frequent symptoms are right upper quadrant pain and dullness in a small number. Mild or moderate hepatomegaly is one of the most frequent findings during the examination. Serum levels of biochemical parameters in patients with NAFLD show hyperlipidemia, hyperglycemia, hyperinsulinemia, and reduced insulin sensitivity. Aminotransferases are increased moderately, and AST/ALT ratio is <1. Inversion of the AST/ALT ratio to >1 may suggest progression to cirrhosis in patients with NAFLD. NAFLD patients with normal aminotransferases may exhibit the full spectrum of histopathological abnormalities, from benign steatosis to cirrhosis. Previously, our group showed that serum aminotransferase values fluctuate during the course of the disease (17). Moreover, there is no good correlation between serum liver tests and necroinflammatory activity or stage (18). Abdominal ultrasonography, computed tomography, and magnetic resonance imaging can show NAFLD but can not separate benign steatosis from NASH (12). However, proton magnetic resonance spectroscopy can measure hepatic triglyceride content.

Table 1. Terminology of nonalcoholic fatty liver disease (NAFLD)

1. NAFLD: Predominantly macrovesicular steatosis
2. Simple steatosis: Benign form of NAFLD
3. NASH: The progressive form of NAFLD that includes necroinflammation, ballooning of hepatocytes, and variable degrees of fibrosis
4. NAFLD-associated cirrhosis: Loss of one of the histological hallmark features of NAFLD (fat)
5. NAFLD-associated HCC

HCC: hepatocellular carcinoma; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis

Table 2. Histopathologic abnormalities in nonalcoholic steatohepatitis (NASH)

1. Steatosis
2. Inflammation
3. Hepatocyte ballooning
4. Fibrosis

PATHOGENESIS OF NAFLD

A growing body of evidence shows that the primary abnormality is a metabolic disturbance in NAFLD (12). Insulin resistance is at the center of this metabolic abnormality and may cause NAFLD. Then, a fatty liver may cause hepatocyte injury and inflammation by oxidative stress and may progress to NASH and cirrhosis (Figure 1).

The main element of NAFLD is the accumulation of triglycerides (TG) as fat droplets within the cytoplasm of hepatocytes, which is a prerequisite for subsequent events of NASH, as more than 5%-10% of hepatocytes have fat droplets, as evident on liver biopsy (4) (Table 2). Increased delivery of both free fatty acids (FFA) and TG to the liver, diminished hepatic utilization of FFA, diminished export of TG from the liver, and impaired beta-oxidation of FFA within hepatocytes cause TG accumulation within the cytoplasm of hepatocytes (12-14). Excess carbohydrate, either from dietary sources or de novo gluconeogenesis in the liver, is also a major stimulus for de novo fatty acid synthesis in the liver.

Obesity is a low-grade chronic inflammatory condition. Obesity-related cytokines, such as interleukin-6 (IL-6), adiponectin, leptin, and tumor necrosis factor-alpha (TNF- α), play important roles in the development of NAFLD. Adipose tissue is now recognized as a source of important inflammatory mediators and adipokines, such as pro-inflammatory (leptin, TNF- α , and IL-6) and anti-inflammatory (adiponectin) effects (13-16). Although these cytokines and hormones may normally work in balance, this homeostasis is disturbed in NASH, such as decreased adiponectin levels and increased TNF- α . Multiple mechanisms may play a role in causing hepatocellular injury in the setting of NAFLD, many of which generate reactive oxygen species (ROS). A liver with excess fat is more vulnerable to stressors, such as reactive ROS, adipokines, and cytokines, than a normal

liver. The regenerative capacity of a fatty liver is also impaired. Yang and colleagues demonstrated that obese mice with fatty liver clear endotoxins less than nonobese controls.

The factors that play key roles in the development of NASH from simple fatty liver remain uncertain (19-24). Some possibilities are oxidative stress by increased ROS and decreased antioxidants, lipid peroxidation, reactive metabolites, such as malondialdehyde and 4-hydroxynonenal, adipose tissue products, transforming growth factor- β_1 , Fas ligand, mitochondrial dysfunction and respiratory chain deficiency, and intestinal microbiota by increased intestinal permeability, intestinal leak, increased energy harvesting from diet, bacterial lipopolysaccharides (LPS), endotoxins, and TNF- α .

INTESTINAL MICROBIOTA: A KEY ROLE IN THE DEVELOPMENT OF NAFLD

The human gut has a highly diverse microbial ecosystem, which includes approximately 400 different species (25). This highly diverse microbial system, as "a bioreactor," has important functions in the host, such as digestion of complex carbohydrates (26,27). So, the intestinal microbiota may contribute to the development of obesity and NAFLD. Today, a growing body of literature reports gut microbiota as a new environmental factor contributing to obesity and NAFLD (28-30).

The human intestine is a neuroendocrine organ and has its own immune system, which includes approximately 70% of the total body lymphocytes. After birth, the intestine is infected by mainly firmicutes (increased capacity of energy harvest from diet, such as butyrate), bacteroidetes (produce acetate and propionate), actinobacteria, and proteobacteria (26-28). Colon bacteria produce short-chain fatty acids from fibers. The microbiota diversity is mainly observed in the adult and less in elderly. The ratio of firmicutes to bacteroidetes is higher in adults than in the elderly. The composition of bacteria in the colon mucosa and feces is different from each other.

The intestinal microbiota has two major functions: metabolic roles and protective roles (26,27). Metabolic roles include improving digestion, such as vitamin synthesis and the metabolism of carbohydrates, lipid, and protein; supplying micro-nutrients; and improving bioavailability. Increased microbial fermentation of short-chain fatty acids (SCFAs), bacterial LPS, and epithelial FIAF (pgc-1 α) increases both hepatic ($\uparrow$ SCFAs) and adipose tissue lipogenesis ($\downarrow$ FIAF and $\uparrow$ LPS, $\uparrow$ ChREBP, $\uparrow$ SREBP-1, $\uparrow$ FAS, and $\uparrow$ ACC) and decreases fatty acid oxidation in the skeletal muscle.

Germ-free (GF) C57BL/6J mice are microbe-free and resistant to diet-induced obesity (31-34). GF mice gain less weight than conventional mice when given a sugar- and lipid-rich diet despite greater food consumption. GF mice receiving a high-fat diet show enhanced insulin sensitivity with improved glucose tolerance and reduced insulinemia. Development of obesity in

Başaranoğlu and Örmeci. Nonalcoholic fatty liver disease

diet-induced obese mice is associated with dramatic changes in the composition and metabolic function of the microbiota. Then, conventionalization of germ-free mice with a normal gut microbiota harvested from the intestine of conventionally raised mice results in total body fat gain and liver weight gain and leads to obesity. These results are gained in less than 10 days and rapid.

Proposed mechanisms for the role of gut microbiota include the provision of additional energy by the conversion of dietary fiber to short-chain fatty acids, effects on gut hormone production, increased intestinal permeability causing elevated systemic levels of lipopolysaccharides (LPS), and the innate immune system, as mice deficient in Toll-like receptor 5 develop hyperphagia and become obese and insulin-resistant (35-38). Recently, a group reported that gut microbiota influences whole-body glucose homeostasis and liver lipid metabolism in mice. Obese mice have fewer bacteroidetes and more firmicutes. Obese humans have also more firmicutes and fewer bacteroidetes. Diet-induced weight loss decreases firmicutes in obese humans.

THERAPY OF NAFLD AND NASH

Nonalcoholic fatty liver disease is the liver component of metabolic syndrome, which includes abdominal obesity, high plasma triglycerides, high fasting glucose, high blood pressure, and low plasma HDL-cholesterol (11,12,19,24,39-44). Metabolic syndrome also covers several disturbances in the body, such as endothelial dysfunction, prothrombotic state, atherogenic dyslipidemia, chronic low-grade inflammation, and insulin resistance (45). Currently, there is no well-established and approved therapy for NAFLD. Primary recommendations are to improve existing co-morbidities, such as obesity, hyperlipidemia, or type 2 diabetes, and lifestyle modification with weight loss and exercise. The most probable death cause is cardiovascular diseases in patients with NAFLD due to cardiovascular involvement in metabolic syndrome. However, liver-related diseases, such as cirrhosis, end-stage liver diseases, hepatocellular cancer, and liver transplantation, are being increased due to the obesity epidemic over time. We must divide patients with NAFLD into 2 groups according to follow-up results: patients with simple steatosis, which is a relatively benign condition, and NASH, in which liver-related mortality is increased (46). In light of the current knowledge, the basic principles for the management of NASH should be to decrease insulin resistance, to reduce visceral adiposity and fat mass, to decrease hyperlipidemia and hypertension, and to reduce oxidative stress and lipid peroxidation products (47).

Lifestyle modification is defined by doing exercise 180 minutes per week, restricting the overconsumption of calories for weight loss, stop smoking and drinking alcohol, and sleeping on time (45). To restrict energy, the patient with NASH should decrease whole grain intake (increasing fiber and Mg intake in diet) and carbohydrate intake with a normal or high protein

Table 3. Potential future pharmacologic therapy modalities in NAFLD

1. Insulin-sensitizers
2. Anti-lipidemic agents
3. Cytoprotectives and antioxidants, such as ursodeoxycholic acid, vitamin E, S-adenosylmethionine, N-acetylcysteine, selenium, carnitine, silymarin
4. Antibiotics and probiotics to reduce gut-derived endotoxins

diet (0.8 g/kg or 1.4 g/kg). As low carbohydrate intake reduces both waist circumference and fasting plasma glycogen, low-fat diet improves plasma LDL-cholesterol and HDL-cholesterol levels (48). Monounsaturated fatty acids (MUFA) and MUFA-rich foods have been found to be protective against metabolic syndrome and its cardiovascular component. However, polyunsaturated fatty acids have no beneficial effect on liver histology, body weight, waist circumference, blood pressure, LDL-cholesterol, and C-reactive protein but improve serum ALT and simple steatosis. Dietary antioxidant supplementation, high dairy intake, daily egg intake, and even probiotics are recommended in patients with NAFLD. It was also reported that physical exercise improves hepatic steatosis, measured by magnetic resonance spectroscopy and plasma ALT levels, besides waist circumference, insulin resistance, and the plasma levels of fasting glucose, HbA1c, LDL cholesterol, and triacylglycerol. Both hepatic steatosis and inflammation are improved by weight loss in a short time. But, the response of fibrosis is very slow.

If the patient's condition does not improve despite these interventions, medications may be considered. Goals for pharmacologic therapy are to decrease accumulation of fat in the liver and consequent injury. Pharmacotherapy options for NAFLD are insulin sensitizers (metformin, thiazolidines), antioxidants (vit E and C), hepatocyte protective agents (UDCA), antiobesity drugs (orlistat), antidyslipidemia agents (statins, fibrates, NPC1L1 inhibitors (ezetimibe)), RAS blockers, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, antialdosterone, GLP-1 agonist (exenatide), and DPP-4 inhibitors (sitagliptin) (48). The gut microbiota might be a new prevention or therapeutic approach for improving obesity and NAFLD (Table 3).

A meta-analysis of studies performed with thiazolidines showed improvements in steatosis, hepatocyte ballooning, and inflammation but not in fibrosis (49-55). Insulin resistance, fasting plasma glucose levels, and plasma levels of HbA1c, HDL-cholesterol, triglyceride, C-reactive protein, and adiponectin levels were also improved by thiazolidine therapy. Metformin is also widely used by NASH researchers. It was reported that waist circumference, body weight, fasting plasma glucose, and adiponectin levels and insulin resistance were all improved, but there was no effect on liver histological findings. UDCA, as a hepatocyte membrane stabilizer, has no effect on liver histology but improves plasma ALT, HbA1c, and HDL-cholesterol levels. UDCA (1000 mg/day) therapy in combination

with vit E (500 mg/day) improved serum liver enzymes (ALT, AST, and GGT) significantly, but the dropout level was 5% due to side effects (47).

Statins were used in 4 randomized controlled trials and showed that there was no effect on liver histology and body weight (56-63). Fibrates have a beneficial effect on liver enzymes in a short period but no effect on liver pathologic scores.

TNF- α levels are increased in both insulin resistance and NAFLD. So, as a TNF- α blocker, pentoxifylline improves both steatosis and inflammation in patients with NAFLD and NASH (64-66). Antioxidants showed modest improvement in steatosis and lobular inflammation, with no effect on body weight, waist circumference, LDL-cholesterol, and fibrosis (48,67).

Traditional Chinese medicines or herbs might play an important role in this field, and some patients benefit from it. Evidence from randomized controlled trials (RCTs) for the efficiency of traditional Chinese medicine (TCM) on the treatment of NAFLD is conflicting. A systematic review and meta-analysis of RCTs to evaluate the efficiency and safety of TCM in the treatment of NAFLD showed that TCM had a better effect on the normalization of ALT and disappearance of radiological steatosis in the treatment of NAFLD and concluded that TCM is of modest benefit in the treatment of NAFLD (68).

Despite the decreasing number of hepatitis C and B virus infections, the number of patients with NAFLD and particularly advanced liver disease (NASH) will be increasing (69-74). We need to decrease the prevalence of the obesity burden and reduce the disease burden (NAFLD and NASH) and related morbidity and mortality in the world.

NAFLD AND NASH AS RISK FACTORS

Nonalcoholic fatty liver disease is a risk factor for a lot of conditions, such as colorectal malignant neoplasm and in-stent restenosis after bare metal stenting in native coronary arteries (75,76).

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