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Consice Review

Nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease is a clinicopathologic syndrome that encompasses several clinical entities. The spectrum of conditions ranges from simple steatosis to steatohepatitis, fibrosis and end stage liver disease. The condition was originally described in obese, diabetic, middle-aged females without a history of significant alcohol use with liver histology consistent with alcoholic hepatitis.[1,2] It is known that this entity occurs without any particular sex predilection, in lean individuals,[3] as well as an increasing number of obese children.[4]

Other terms have been used to describe this clinical entity such as alcohol-like hepatitis, pseudo-alcoholic hepatitis, diabetic hepatitis and steatonecrosis. Ludwig and colleagues introduced the term nonalcoholic steatohepatitis (NASH) to describe patients fitting the picture of alcoholic hepatitis but without a history of significant alcohol abuse.[2] The term nonalcoholic fatty liver disease (NAFLD) is used more frequently to include the spectrum of conditions that range from steatosis through steatohepatitis, fibrosis and cirrhosis. NASH is reserved for patients with steatohepatitis and fibrosis.

NAFLD is now being recognized as the most common cause of elevated liver enzymes in the United States. Although the exact etiology of NAFLD is not known, it may be caused by insulin resistance coupled with increased oxidative stress to the hepatocytes. No specific therapy has been approved for this condition and the mainstay of management is weight loss.

Key words: Nonalcoholic steatohepatitis, liver, obesity, fatty liver.

Introduction

Nonalcoholic fatty liver disease is often associated with obesity, type II diabetes mellitus and dyslipidemia, and is often regarded as the hepatic manifestation of the metabolic syndrome-the condition of dyslipidemia, hypertension, obesity and diabetes.[5] Disease severity may be classified according to histologic findings. Some patients with NAFLD have simple hepatic steatosis without any associated inflammation or fibrosis. Others, with the more advanced form, have a combination of steatosis with either/or nonspecific inflammation, ballooning degeneration of the hepatocytes, fibrosis and Mallory bodies. This latter group is classified as NASH.[6,7]

It must be acknowledged that there are other secondary causes of hepatic steatosis such as drugs (corticosteroids, amiodarone, synthetic estrogens, etc.), rapid weight loss, surgical procedures (jejunoileal bypass or extensive small bowel resection) and miscellaneous causes such as TPN and diverticulosis,[7] *(see table I)*. This review will focus on nonalcoholic fatty liver disease without any other associated factor.

Epidemiology

NAFLD is a worldwide phenomenon spanning all the continents.[7] The exact prevalence is uncertain. In one study, 126 candidates for adult-to-adult living donor liver transplant underwent liver biopsy as part of the pre-transplant evaluation. Twenty percent were found to have fatty liver which made them ineligible donors.[8] Another study that reviewed liver histology of 503 consecutive fatal traffic accident victims and 423 aircrew fatalities, found that the prevalence of steatosis (NAFLD) was 24% and 15.6% respectively. In this series, the prevalence of steatosis associated with inflammation and fibrosis (NASH) was 3%.[9,10]

Over the last several decades, obesity – defined as body-mass index $\geq$ 30 kg/m^2, has emerged as a major medical problem,[11,12] Current data indicate that the prevalence of obesity among US adults was 20.9% in 2001 (a 74% increase since 1991). The prevalence of those with diabetes increased to 7.9% in 2001(a 61% increase since 1990).[13] These numbers are believed to underrepresent the true extent of the problem. We know that obesity is often associated with insulin resistance, diabetes, and hyperlipidemia.[14,15] One autopsy series of 351 non-alcoholics found a prevalence of steatohepatitis of 2.7% in lean versus 18.5% in markedly obese patients. Severe fibrosis was found in 6.6% of lean and 13.8% of markedly obese individuals. This may be explained by the higher prevalence of diabetes in the obese group.[16] Other published series note a prevalence of NAFLD of 40 to 75% in obese persons.[16,17] Likewise, prevalence of diabetes ranging

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from 40% to 75% has been reported in patients with NASH.[6,18] Given the close association of NAFLD with obesity and diabetes, this substantial increase in their prevalence portends an increase in the cases of NAFLD in the future. One must bear in mind however, that populations other than obese, diabetic, middle-aged women are at risk for developing steatosis and NASH. This was demonstrated in a study of the clinical profile of a group of patients with NASH. Of those studied, 19 of 33 patients (58%) were male and 26 of 33 patients (79%) had normal glucose levels.[3]

In addition, the increase in the number of cases of NAFLD in children and adolescents appear to be in parallel to the adult condition since the earliest reported case in 1983.[19,20] Interestingly, it is noted that NAFLD is more frequent in young boys than girls and tends to occur where there is a family history of diabetes and obesity.[4]

Clinical presentation

Most patients with NAFLD are asymptomatic. Many are discovered when elevated transaminases are detected on routine laboratory tests or during evaluation for other conditions such as obesity, diabetes, hyperlipidemia or hypertension. Symptoms are usually non-specific when they occur. Some patients have fatigue, malaise, or vague right upper quadrant pain. Obesity is the most common finding on physical exam and hepatomegaly is the most common sign of liver disease.[21] Stigmata of chronic liver disease such as spider nevi, palmar erythema, jaundice, gynecomastia, asterixis and muscle wasting are typically not seen until the later stages of the disease.

Diagnosis

There are a number of prerequisite criteria to fulfill in order to entertain the diagnosis of NAFLD. As the name implies, patients under consideration must lack a history of significant alcohol use. Studies show that as little as 20 to 30 g of daily alcohol intake can lead to alcohol induced liver disease.[22,23] In addition, one must ideally rule out other primary liver diseases or any other secondary causes of NAFLD. In some patients, NAFLD or NASH may be superimposed on other liver diseases (i.e. viral hepatitis, autoimmune liver disease).

Table I. Secondary causes of hepatic steatosis.

Surgical procedures	Jejunoileal bypass
	Extensive small bowel resection
Drugs	Amiodarone
	Choroquine
	Perhexiline
	Corticosteroids
	Synthetic estrogen
Medical conditions	Inflammatory bowel disease
	Weber-Christian disease
	Acute fatty liver of pregnancy
Miscellaneous	TPN
	Diverticulosis
	Rapid weight loss

Laboratory assessment

No single laboratory test can diagnose NAFLD or accurately differentiate between the different stages from steatosis to full-blown NASH. The most common laboratory abnormality is an elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[24] The elevation rarely exceeds 2 to 3 times the upper limits of normal values. The pattern of aminotransferase elevation may be helpful to distinguish NAFLD from alcoholic liver disease. The ALT/AST ratio is usually < 1 in NAFLD and in alcoholic liver disease AST is predominantly elevated. An AST/ALT ratio > 1 is more indicative of fibrosis and advanced disease. Generally the alkaline phosphatase and gamma-glutamyl transferase (GGT) are normal or mildly elevated. Serum albumin, prothrombin time and bilirubin are usually normal until the later stages of the disease.

Radiologic assessment

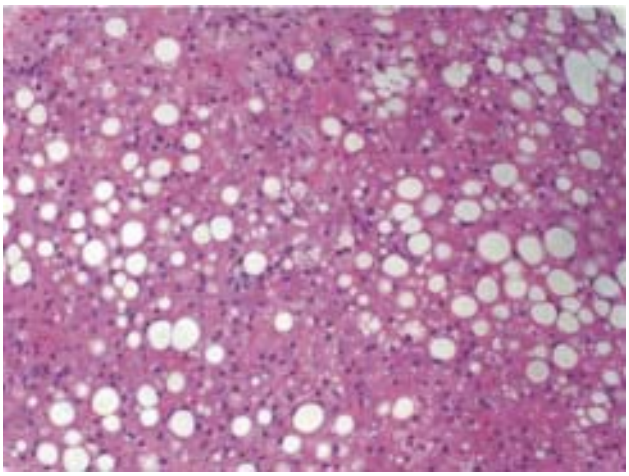
Current routine imaging studies (ultrasonography, computed tomography, and magnetic resonance imaging) are reliable, non-invasive methods of evaluating fatty liver disease.

Criteria for detecting the fatty liver on ultrasound include the brightness of the liver in comparison to the kidney (liver kidney contrast), vascular blurring (obscuring the hepatic vein trunk) and deep attenuation (intensity of the echo beam in the right hepatic lobe). With well applied and standardized ultrasonographic criteria, fatty liver was diagnosed with a sensitivity of 83% and a specificity of 100%.[25] However, this required fatty changes in at least 30% of the liver.

Computerized tomography (CT) imaging determines the presence of fatty liver when the liver minus the spleen attenuation is less than -10 Hounsfield units. Based on this criterion, unenhanced CT remains the most accurate method for detecting steatosis.[26]

One study found that unenhanced CT had a sensitivity of 93% and a positive predictive value of 76% when one third of the liver had fatty infiltration.[27]

MRI takes advantage of the characteristic differences in resonant frequencies between fat and water. Signal intensity is at a maximum when the signals of fat and water are in phase. As such, the fatty liver has higher signal intensity on in-phase images and a loss of signal intensity on out-of phase image.[28] When compared to the spleen, the fatty liver appears darker on magnetic resonance imaging. MRI may more readily differentiate between focal fat versus a focal mass, otherwise it offers no advantage



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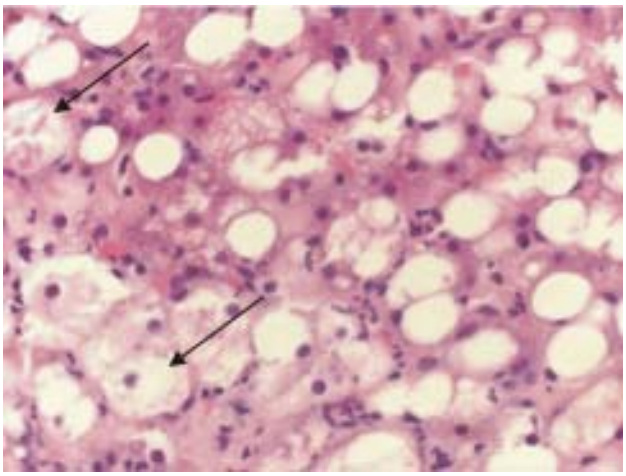

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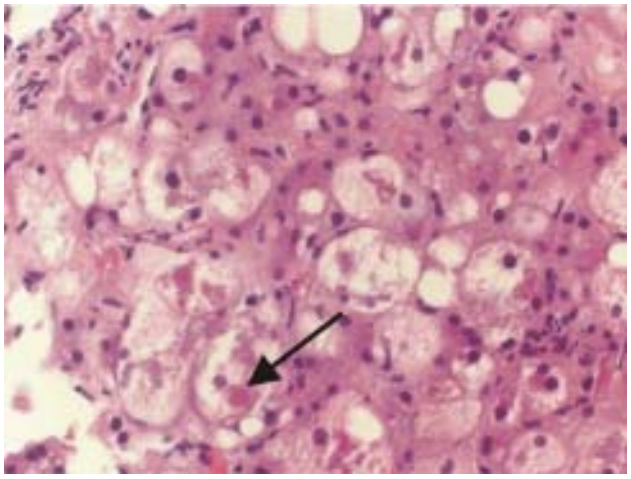

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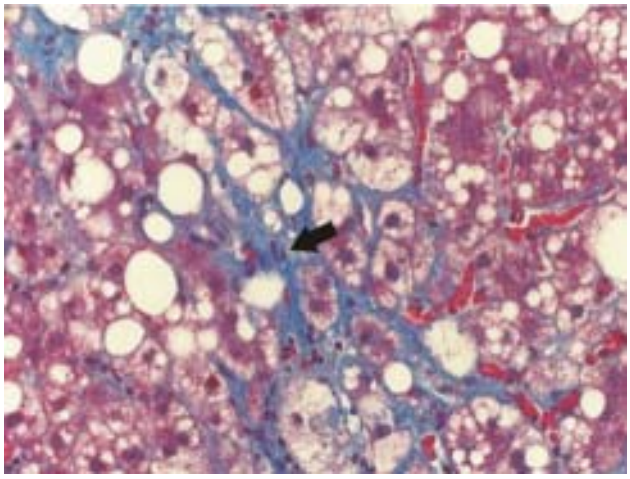

D.

Figure 1. Hematoxylin and eosin stain showing predominantly macrovesicular steatosis **(A)** and an enlarged view showing steatosis, ballooning changes (arrows), and lobular inflammation **(B)**. **(C)** Hematoxylin and eosin stain highlighting Mallory bodies (arrows). **(D)** Masson trichrome stain highlighting extensive sinusoidal fibrosis (arrowhead). *Slides courtesy of Hanlin L. Wang, MD, PhD. Asst Professor. Dept. of Pathology & Immunology, Washington University School of Medicine.*

Demographic	Steatosis	NASH
General population	16-23%	2-3%
Obese	65-85%	19%
Severely obese (BMI $\geq$ 40 kg/m^2)	> 90%	~ 50%
Diabetic	21-78%	35%

Prevalence estimates of steatosis and NASH in various populations.

	Steatosis	NASH
Obese – 38 million with NAFLD	29.6 million	8.4 million
Diabetes – 7.8 million with NAFLD	4.0 million	3.8 million

Population projection for the year 2020 if obesity and diabetes trends continue.

over CT imaging and is less readily available and more expensive.

Magnetic resonance spectroscopy (MRS) is the only imaging modality that offers some quantitative measurement of hepatic fat content. In one study, MRS was shown to correlate well with histologic grading of fatty infiltration of the liver.[29] In another study, MRS accurately assessed hepatic triglyceride content in 15 patients.[30] MRS is not readily available for routine clinical use; its accuracy and reproducibility improve when fatty infiltration is diffuse throughout the liver.

In recent years, attempts have been made to directly evaluate the utility of radiological imaging in the evaluation of NAFLD. When ultrasonography, CT and MRI

were directly compared in patients with biopsy-proven NAFLD, no modality was able to distinguish only steatosis from more advance stages that include inflammation or fibrosis. Steatosis was the only pathological feature detected radiologically.[27]

Pathology

The histologic examination of the liver remains the gold standard for NAFLD diagnosis.

The histopathologic lesions required for the diagnosis of NASH include; macrovesicular steatosis and one or all of the following: a mixed, lobular inflammatory infiltrate, hepatocellular ballooning, typically in zone 3. The following features are supportive but are not absolutely necessary for the diagnosis: perisinusoidal fibrosis and Mallory's hyaline in zone 3.[31]

Some variability in the interpretation of histologic parameters still exists. One study addressed this issue in a review of 19 histologic features of NAFLD. Four liver pathologists reviewed liver biopsy specimens of patients with NAFLD. The interobserver and intraobserver concordance were documented. Moderate to substantial concordance for features such as extent of steatosis, location and grade of fibrosis, ballooning degeneration, hepatocyte necrosis and Mallory bodies was found. There was poor concordance in the assessment of inflammation.[32] In an effort to standardize the evaluation, a grading and staging system of the histologic features of NASH has been proposed. This system takes into consideration the grade of inflammation as well as the stage of fibrosis.[33] Grading of inflammation considers such variables as macrovesicular steatosis, hepatocytes ballooning, lobular versus portal inflammation and Mallory's hyaline. Fibrosis was staged according to the extent of perisinusoidal, portal or bridging extension.

Natural history

The natural history of NAFLD varies according to histologic stage. NAFLD is generally regarded as a condition with an indolent course.

One study followed the course of 40 patients with steatosis only, over a median of 11 years. None had progressed to cirrhosis (one progressed to fibrosis) and none died of liver-related illnesses.[34] In other instances, up to 16% of patients with NAFLD present with more advance stages, with either fibrosis or cirrhosis.[1,3,18,35] Outcome is generally worse for patients detected with fibrosis, ballooning degeneration or necrosis on liver biopsy. In one study comparing patients with fatty liver and inflammation versus fibrosis and necrosis, more subjects in the latter group developed cirrhosis (24% *versus* 3%) and liver-related deaths (30% *versus* 5%).[36] Therefore, it is important to develop methods to detect or predict which patients may have a benign course versus those at risk for progression to end stage liver disease. Reliable prediction may also help to determine when to perform a liver biopsy, when to start "specific" management or establish an accurate prognosis.

In an attempt to identify predictors of fibrosis, one group investigated 144 patients with NAFLD. Older age, obesity, diabetes and ALT/AST ratio > 1 were significant predictors of severe liver fibrosis.[35] In another study, insulin resistance and hypertension were independent predictors of advanced NAFLD in severely obese subjects.[37] This subgroup of patients may benefit the most from a liver biopsy.

The incidence of hepatocellular carcinoma (HCC) has increased in the United States. This has been attributed to its association with hepatitis B and hepatitis C infection.[38] However, with the increased incidence of obesity and diabetes, NAFLD may emerge as an important precursor to cirrhosis and therefore, HCC. One recent study examined the role of NAFLD in the development of HCC. Of 105 consecutive patients with HCC, 13% had a prior diagnosis of NASH or clinical features consistent with NAFLD.[39] Another series followed 82 NASH patients over a decade. About 7% had progressed to cirrhosis and HCC.[40] A recent retrospective analysis of patients with HCC, found that 4% (half of those with cryptogenic cirrhosis being followed at study time) had features consistent with NAFLD.[41] Larger prospective studies are necessary to validate the association of NAFLD and HCC. If the results of the initial smaller studies are validated, routine screening for HCC may be indicated for patients with cirrhosis secondary to NASH.

Pathogenesis

The pathogenesis of nonalcoholic fatty liver disease remains poorly understood. It is a complex, multifactorial process that involves genetic and environmental elements.[42,43]

In 1998, a "two-hit" hypothesis was proposed to explain the pathogenesis of NAFLD, based on existing human and animal studies.[44] According to this proposal, the first "hit" is the accumulation of fat in the liver. Once the liver shows fatty infiltration, a second "hit" triggers the progression from steatosis to steatohepatitis. Initially the first hit was considered to be insulin resistance. Recently, this concept has been expanded to also include unknown genetic factors and obesity.[45]

Individuals with a genetic predisposition develop obesity (visceral or total body obesity) and subsequently insulin resistance. Insulin resistance and hyperinsulinemia, through a complex cascade of events, result in increased peripheral lipolysis with delivery of free fatty acids (FFA) to the liver and increased endogenous hepatic synthesis of fatty acids. Hyperinsulinemia appears to decrease mitochondrial β-oxidation of fatty acids and VLDL synthesis and secretion.[5,46] The net result is liver

steatosis (first "hit") that sensitizes the liver to oxidative stress (second "hit").

Several agents have been identified as contributors to this oxidative stress: mitochondrial source of reactive oxygen species (ROS), increased lipid peroxidation, increased activity of the cytochrome P450 enzyme CYP2E1 and the effects of cytokines such as tumor necrosis factor alpha (TNF α), transforming growth factor beta (TGF-β) and interleukin 8 (IL-8).

Patients with NASH have been found to have structural mitochondrial defects with associated deficiencies in the electron transport chain as well as increased lipid peroxidation levels.[47] In this setting of mitochondrial abnormality and increase hepatic lipid FFA (per the mechanisms described above), there is increased production of ROS leading to lipid peroxidation. Lipid peroxidation products impair the flow of electrons along the respiratory chain, which increases ROS and the cycle repeats indefinitely.[45,48] Cytokines have been implicated in increased oxidative stress as well. Reactive oxygen species and lipid peroxidation products contribute to stimulating the release of inflammatory mediators such as TNF-α and TGF-β and IL-8 that in turn promote neutrophil infiltration, fibrosis, Mallory bodies and hepatocyte death.[44]

Fatty acids are inducers of CYP2E1. One study demonstrated an increased in the expression of CYP2E1 in livers of patients with NASH. The pattern of distribution was predominantly around the terminal hepatic vein (zone 3); similar to the pattern of injury typically seen in NASH.[49] Increased activity of CYP2E1 may result in oxidative stress through direct hepatocyte injury or recruitment of inflammatory cells.[46]

The pathogenesis of NAFLD remains highly speculative and our understanding of the above-described mechanism is evolving. Elucidating the pathway from steatosis to inflammation, fibrosis and cirrhosis, will help in designing treatment protocols targeted at specific sites of pathogenesis.

Treatment

There is no established pharmacological treatment for NAFLD.

The effects of weight loss in patients with NAFLD remain conflicting. In one study, 41 extremely obese patients were monitored before and after losing a median of 34 kg. Fatty changes in the liver and liver biochemistry improved. However, 24% of the patients developed mild portal inflammation or fibrosis (only in the group with rapid weight loss).[50] In a retrospective study of 39 overweight patients, weight loss corresponded with improvement in biochemical markers. No histologic data was available in this population.[51] The available information supports the idea of gradual weight loss (initial goal: 10% of total body weight at six months), averaging about 1-2 pounds per week.[52,53]

Other treatment protocols focus on treating the associated conditions such as diabetes and hyperlipidemia. However, control of diabetes and hyperlipidemia do not result in reversal of NAFLD.

A small number of pilot studies have attempted to evaluate the role of pharmacotherapy in controlling or reversing NAFLD. Drugs studied include ursodeoxycholic acid (UDCA), clofibrate, vitamin E, metformin, betaine, and N-acetylcysteine.

UDCA is a hydrophilic dihydroxy bile acid. It has several properties that make it a desirable agent in the treatment of liver disease. UDCA variably decreases the concentration of toxic endogenous bile acids, it has immunomodulatory effects and it is protective to hepatocytes.[54] In one open-label trial, 24 patients were treated with 13-15 mg/kg/d of UDCA for twelve months. Most patients had a significant decrease in serum alkaline phosphatase, ALT, and GGT, with improvement in the grade of hepatic steatosis.[55] Similar liver chemistry results were found in two studies where patients were treated with UDCA 10 mg/kg/d for six months.[56] Despite these good biochemical outcome, unfortunately, there was no histologic data analysis in these two studies. The long-term benefit of UDCA is currently being investigated.

Clofibrate is an agent used for the treatment of hyperlipidemia associated with high triglyceride levels. In one study, 16 patients with NASH and hypertriglyceridemia were place on Clofibrate 2 gm/d for twelve months. There was no significant change in serum liver tests compared to baseline at the time of entry into the study.[55]

Vitamin E has been observed to reduce oxidative stress, hence the rationale for treatment in NAFLD. In one small, open-label pilot study, 11 children diagnosed with NASH were treated with Vitamin E: 400 and 1,200 IU daily. Serum aminotransferase levels decreased significantly during the 4-10 month study period, but returned to abnormal levels when patients elected to discontinue the medication.[57]

Metformin is an agent that improves hepatic insulin sensitivity. Therefore it is not surprising that it has been seen as a likely candidate for treatment of NAFLD, where insulin resistance is known to occur. Metformin reversed hepatomegaly, steatosis and abnormal aminotransferase in obese, leptin-deficient mice.[58] In one open-label trial, 20 patients with NASH were treated with metformin 500 mg three times daily for 4 months. Six patients did not complete the trial and were used as controls. Those in the treatment arm experienced improvement in hepatic insulin sensitivity, significant reduction in aminotransferase levels (normalized in 50%) and 20% reduction in hepatomegaly.[59] Trials involving metformin and other hypoglycemic agents are currently underway.

Betaine is a nutrient that functions closely with others such as S-adenosylmethionine (SAME), folic acid and vitamins B6 and B12 to lower levels of homocysteine. Seven of ten patients completed a study in which betaine an-

hydrous solution 20 g/d was administered for 12 months. Six of seven patients had significant improvement in aminotransferase levels. The degree of steatosis, fibrosis and necroinflammatory grade also improved.[60] In a larger prospective randomized double-blinded trial, 96 patients were treated with betaine glucoronate, diethanolamine glucoronate and nicotinamide ascorbate, while 95 patients were randomized to placebo. There was significant reduction in serum AST, ALT and GGT in the treatment group. In addition, there was a 25% reduction in steatosis and 6% reduction in hepatomegaly in those treated.[61] This study included more patients but is limited by a short treatment duration and the lack of histology.

N-acetylcysteine (NAC) is a glutathione precursor that increases the stores of glutathione and act as a substrate for toxic metabolites. Preliminary results of one study of 11 patients treated with NAC 1 g/d for 3 months, showed improvement in aminotransferase levels.[62] As with some other studies, this was of a short duration with few patients and no histologic data. Therefore, validation through larger studies is needed.

Other classes or drugs such as the thiazolidinediones have been studied, revealing promising preliminary results. However, further large controlled studies are needed before recommendations can be issued.

Cryptogenic cirrhosis is the third leading cause of orthotopic liver transplant (OLT) in the United States (based on OPTN data as of October 2003).[63] It is now proposed that up to half of the cases designated as cryptogenic, may indeed be attributed to NASH cirrhosis. However, the precise number of patients undergoing orthotopic liver transplant secondary to NASH induced liver failure remains uncertain. This may be explained in part, by the fact that some patients with end-stage NAFLD may have a "burned out" histology and so are sometimes classified as unknown etiology or cryptogenic cirrhosis.[64] In addition, earlier cases of NASH may have gone unrecognized due to a lack of familiarity with this clinicopathologic syndrome. Therefore, studies evaluating the relationship between NASH and liver transplantation are mostly retrospective and rely on clinical syndrome correlation for the "diagnosis" of NASH. Two studies illustrate this point. One study reviewed 27 patients with cryptogenic cirrhosis and a "clinical histological phenotype of NASH" and 3 patients with a known diagnosis of NASH. After a five-year follow up, all 27 assessable patients (1 patient died perioperatively, 2 died later of sepsis) had recurrent steatosis in the allograft (compared to 25% in the control group with pre-OLT diagnosis of primary biliary cirrhosis and primary sclerosing cholangitis). Three patients had progression from steatosis to steatohepatitis (one of these developed progressive fibrosis).[65] Another series identified eight patients (of 622 cases) transplanted for NASH. Subjects were followed for up to four years. Six patients had recurrent fatty liver (3 with NASH).[66] Orthotopic liver transplantation may play an increasing role in the management of NAFLD. Even though it is not a direct treatment for NAFLD, but a salvage strategy for those who progress to liver failure.

In Summary non alcoholic fatty liver disease encompasses a wide spectrum of disorders. Those with simple steatosis tend to remain stable over many years. However, those with the more advanced form of the disease (steatosis with inflammation and fibrosis) tend to progress more rapidly to cirrhosis and end stage liver disease. Its association with type II diabetes mellitus and obesity portend an epidemic of NAFLD, as the number of obese and diabetic patients is increasing at alarming rates. Therefore, it is important to develop reliable, non-invasive diagnostic tools and predictors of the disease to determine which patients will be in need of more aggressive intervention. Initial pilot studies show some promise of treatment options. However, in order to make generalized recommendations about treatment and prognosis, larger well-controlled clinical trials of prolonged duration are necessary.

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