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THERAPY OF NONALCOHOLIC FATTY LIVER DISEASE: CURRENT STATUS

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Nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome, has become a common entity in clinical practice. In most of the patients it presents as simple steatosis with nonprogressive clinical course. However, some patients have progressive form of NAFLD, nonalcoholic steatohepatitis (NASH), and are at increased risk of developing cirrhosis and hepatocellular carcinoma. NAFLD treatment includes lifestyle modifications and pharmacotherapy aiming at increasing insulin sensitivity, and attenuating inflammation and hepatic fibrosis. Weight reduction has consistently been shown to reduce levels of liver enzymes and insulin resistance. Although dietary intervention and exercise remain the first-line therapy, due to low patients compliance to these measures pharmacotherapy or surgical approaches are often required. Metformin and thiazolidinediones may improve insulin sensitivity, serum aminotransferase level and liver histology. However, little evidence exists regarding their sustained effects after drug discontinuation which, together with their side effects, limits their widespread use in clinical practice. Statins appear to be safe agents for the treatment of hyperlipidemia, although trials documenting their efficacy in NAFLD are scarce. Based on the recent clinical trials, weight loss medication orlistat, ursodeoxycholic acid and antioxidant agents could potentially be used as adjunctive therapy. Considering still largely controversial clinical data regarding pharmacological agents, their high cost and known side-effects, lifestyle modifications at present remain the only essential considerations in the NAFLD treatment.

Key words: *nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, treatment, obesity, metformin, thiazolidinediones, fenofibrate, statins, angiotensin receptor blockers, ursodeoxycholic acid, vitamin E, polyunsaturated fatty acids*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, affecting up to 20% of the population in Western countries, and 70-80% of obese individuals (1, 2). Incidence of NAFLD is increasing due to ongoing epidemic of its two major risk factors, obesity and type two diabetes, due to sedentary lifestyle and poor dietary choices. A fact that 90% of patients with NAFLD have at least one characteristic feature of metabolic syndrome and about 33% have the diagnosis of metabolic syndrome, determines NAFLD as hepatic manifestation of metabolic syndrome (3). It encompasses a spectrum of distinct histological entities with different natural history and outcome, ranging from simple fat accumulation in hepatocytes to liver steatosis accompanied with necroinflammatory component that may have associated fibrosis. Simple steatosis is defined as a benign form of NAFLD with minimal risk of progression in contrast to nonalcoholic steatohepatitis (NASH), which progresses to cirrhosis in up to 20% of patients and can subsequently lead to liver failure or hepatocellular carcinoma (4). Rationale for investigating different potential NAFLD treatment modalities comes from understanding the proposed mechanisms in the pathogenesis of the disease. As the currently accepted theory suggests, the first step in disease genesis is liver fat accumulation induced by

changes in lipid metabolism favoring excessive triglyceride accumulation in hepatocytes, as a result of insulin resistance. The second step is believed to be increased oxidative stress within the hepatocytes, which is characterized by excessive production of reactive oxygen species by mitochondria and cytochrome P-450 system in the liver. Subsequently, reactive oxygen species through lipid peroxidation, pro-inflammatory cytokine induction, and Fas ligand induction promote progression from steatosis to steatohepatitis, fibrosis and finally cirrhosis (5-8). Therefore, most of the investigated medical regimens for NAFLD have targeted insulin resistance, components of the metabolic syndrome (primarily obesity and dyslipidemia), and oxidative stress.

INITIAL APPROACH – DIET, WEIGHT LOSS AND EXERCISE

It is well accepted that simple lifestyle modifications such as body weight management with appropriate nutritional counseling, with or without regular physical exercise and cognitive-behavior programs should be the mainstays of therapy for patients with metabolic syndrome. It has been shown that obesity together with insulin resistance, type 2 diabetes and dyslipidemia is a central risk factor for NAFLD, and visceral

adipose tissue has an important role in secretion of several adipokines and cytokines which cause systemic and hepatic insulin resistance, as well as hepatocyte injury and apoptosis, neutrophil chemotaxis, and hepatic stellate cell activation (9, 10). Body weight reduction on the other hand leads to the loss of the adipose tissue, which further leads to the improvement in peripheral and hepatic insulin sensitivity and prevention of hepatic injury. Specific diet and exercise guidelines for NAFLD patients haven't been published, but there are several types of diets which have been proposed for treatment of obesity and metabolic syndrome by different medical and commercial sources (11-19). Most of the published studies in NAFLD population have been consistent with notion that gradual weight loss with a calorie-restricted diet and concomitant exercise leads to a decrease in the incidence of metabolic syndrome, improvement in liver biochemical tests, and resolution of hepatic steatosis (20-24). Rapid weight loss can however aggravate the underlying liver disease causing the portal inflammation and fibrosis, and moderate and graduate weight loss not exceeding loss of 1.6 kg/week with incorporated regular physical aerobic activity regimen including at least 30 min of exercise three times per week is therefore advocated (25). According to the published results, the weight reduction of 5% or more accompanied by regular exercise for one year has been associated with improvement and normalization in liver tests, and keeping this weight (<5% gain) for two consecutive years maintained normal ALT levels (26). Huang *et al.* showed that intense dietary intervention can achieve a reduction in mean waist circumference, insulin resistance, levels of fasting glucose, triglycerides and liver function tests, as well as an improvement in liver histology in patients with NASH (27). Home-based lifestyle modification intervention carried out in collaboration with interdisciplinary medical staff should be the preferred approach in obese NAFLD patients (28). However, there is a problem that the positive effect is commonly short lived, implementation of these lifestyle changes is not always successful, particularly in very obese patients, and long-term data on the liver histological improvement are still lacking. In contrary to the most of published results, study by Larson-Meyer *et al.* showed that there is significant reduction of liver lipid content (measured by magnetic resonance spectroscopy) with caloric restriction (with or without exercise), but without significantly correlated reduction in triglycerides, high-density lipoprotein (HDL)-cholesterol, or ALT levels as well as markers of systemic inflammation (29). Pharmacological treatment for obesity may be offered to those patients with a BMI greater than 30 kg/m² who had no change in the course of disease after adequate lifestyle changes have been undertaken. Orlistat (inhibitor of pancreatic and gastric lipase), was evaluated in a pilot study which included ten obese NASH patients treated for 6 months with this agent (30). Results showed that 10% or greater consequent body weight reduction lead to improvement in aminotransferase levels, liver steatosis and fibrosis. In another double-blind randomized placebo-controlled trial group of patients with NAFLD treated with orlistat for 6 months had significant 2-fold reduction of serum ALT levels and a statistically significant reversal of liver steatosis detected by ultrasound (31). Third study in obese NAFLD patients confirmed a histological improvement in liver fibrosis and inflammation after treatment with orlistat as well as improvement in insulin resistance index and reduction of aminotransferases, total cholesterol, triglycerides and low-density lipoprotein levels (32). Sibutramine is a specific inhibitor of norepinephrine, serotonin and dopamine reuptake into nerve terminals which leads to increased satiety, inhibition of food intake and possibly elevation in energy expenditure. A 6-month study of sibutramine in the obese subjects with NASH

and concomitant low calorie diet showed reduction of body weight and insulin resistance, with improvements in biochemical markers and ultrasound findings of NASH (33). Results of these studies are interesting but there are few concerns which should be addressed. Long-term safety profile of both of these agents is unknown. Orlistat causes gastrointestinal side-effects and malabsorption of fat-soluble vitamins in up to 30% of patients, and sibutramine can increase blood pressure levels (34, 35). Although the mechanisms of action of these two agents do not overlap, their combination is not recommended because there is no proven additive weight loss when used together, and the rate of combined side-effects is unacceptable (36). Bariatric surgery offers advantage of effective long-term weight management in patients with severe obesity. It is associated with improvement in diabetes mellitus, hypertriglyceridemia, hypertension and obstructive sleep apnea and is possible the best therapeutic modality in NAFLD patients with severe obesity (37, 38). Older or 'classical' procedures such as jejunio-ileal bypass have been almost completely replaced by 'newer' procedures such as proximal gastric bypass and biliopancreatic diversion. The reason is the high rate of postoperative complications reported with 'classical' procedures, even though cases of NASH progression and subacute liver failure have also been reported with 'newer' methods (39, 40). Improvement in diabetes mellitus, liver function tests and liver histology (inflammation and fibrosis) was reported in several trials of severe obese NASH patients treated with gastroplasty (41-44), and similar results were reported after gastric bypass surgery (45). Nevertheless, the decision when and in which patients to perform bariatric surgery should still be highly individual. Liposuction is nowadays a commonly used procedure for removal of subcutaneous fat tissue. It offers the advantage of significant weight and fat loss but does not improve peripheral insulin sensitivity and doesn't affect the levels of adipokines (46). Therefore it cannot be recommended as a treatment modality for obese NAFLD patients. Specific recommendations regarding smoking and minimal to moderate alcohol intake in patients with NAFLD cannot be made clearly. On the other hand, cessation of smoking should be one of the aims of primary prevention of metabolic syndrome conditions with an increased risk of mortality, especially in patients at a high risk for cardiovascular disease (47-48).

INSULIN SENSITIZING AGENTS

Metformin is a biguanide, a class of oral hypoglycemic drugs with insulin-sensitizing properties which acts through decreasing the hepatic glucose output, increasing the insulin-mediated glucose utilization in peripheral tissues, and lowering the serum free fatty acid concentrations (49-51). It is considered as the first choice in oral treatment of type 2 diabetes for several reasons: it promotes modest weight reduction in contrast to other hypoglycemic agents, it is less likely to cause hypoglycemia, it has a lipid-lowering activity, and has also a low market cost (52). Animal studies have reported that metformin reverses hepatomegaly, steatosis and liver tests abnormalities in animal models of NAFLD (53). Several smaller clinical studies initially reported improvement in mean serum aminotransferase levels and insulin resistance after 6 months of treatment; although after 1 year of treatment there was no clear effect on aminotransferase levels, liver histology, or insulin sensitivity (54-56). In an open-label randomized study of 110 non-diabetic NAFLD patients, higher rates of ALT normalization and improvement in liver histology and insulin sensitivity were associated with metformin treatment in comparison to vitamin E treatment or weight loss (57). However, some recent open-label randomized studies have

found no benefit of metformin treatment on liver steatosis, assessed either histologically or by CT, aminotransferase levels, or markers of insulin resistance and inflammation in comparison to placebo or lifestyle interventions (58-61). Therefore, it cannot be recommended as an initial treatment for non-obese or obese NAFLD patients, although it still has a role in a treatment of group of NAFLD patients with impairment in glucose or lipid metabolism.

Thiazolidinediones (pioglitazone, rosiglitazone) are novel class of oral antidiabetic drugs that ameliorate insulin sensitivity in euglycemic and diabetic patients by activating peroxisome proliferator-activated receptor (PPAR) gamma (62). In a recent animal study, it has been demonstrated that preventive pioglitazone effects in NASH depend on adiponectin upregulation and subsequent inhibition of sterol regulatory element-binding protein 1c (SREBP-1c), which controls the expression of nearly all genes required for de novo synthesis of fatty acids and triglyceride synthesis (63). Adiponectin has been identified as a pivotal mediator for thiazolidinediones' effects on glucose homeostasis, insulin sensitivity and lipid metabolism (64). It increases fatty acid β -oxidation in muscle, decreases hepatic lipid content in ob/ob mice and has direct anti-fibrotic and anti-inflammatory properties (65-70). In studies with animals, pioglitazone and rosiglitazone prevented activation of hepatic stellate cells *in vitro* and improved hepatic steatosis and prevented liver fibrosis *in vivo* (71, 72). In another animal study, 1 week treatment with pioglitazone significantly decreased hepatic triglyceride content and serum levels of TNF- α and after 4 weeks treatment ameliorated degree of hepatic fibrosis with a decrease levels of procollagen, alpha-smooth muscle actin and TGF- β (73). Rosiglitazone also improved liver enzymes and ameliorated degree of hepatic fibrosis in nutritional fibrosing steatohepatitis (72). First prospective human study included 18 nondiabetic patients with biopsy-proven NASH treated with pioglitazone (30 mg daily) for 48 weeks, reporting improvement in degree of insulin sensitivity, serum alanine aminotransferase normalization and liver histological improvements in hepatic steatosis, cellular injury, parenchymal inflammation, Mallory bodies, and fibrosis (74). Although MRI confirmed a marked decrease in liver fat content and liver volume, main side effect was weight gain, which averaged 3.5 kg and an increase in total body adiposity. In larger prospective controlled trials, treatment with thiazolidinediones led to metabolic, biochemical and histological improvement, including steatosis and ballooning, without significant change in hepatic fibrosis (75-76). In contrast, in a study by Aithal *et al.*, pioglitazone (30 mg/day) treatment for 12 months resulted in improvements in metabolic, biochemical and histological parameters, particularly in liver injury and fibrosis (77). Histological improvements have also been observed in a study of 10 patients treated with combination of pioglitazone (30 mg daily) and vitamin E (400 IU daily) or vitamin E alone (78). Side effects such as fatigue, mild lower extremity edema and weight gain are unfavorable effects that have been reported in patients receiving thiazolidinediones treatment. Answer could be treatment with low-dosages. In a small trial including 12 biopsy-proven NASH patients, low-dose pioglitazone (15 mg/day) administered for 24 weeks improved NASH (79). In almost all aforementioned studies, beneficial effect of thiazolidinediones on the metabolic, biochemical and histological parameters after medication discontinuation has been an important issue. Neuschwander-Tetri *et al.* observed that liver enzymes and glycemic control reverted to pre-treatment values 6 months after discontinuation of rosiglitazone (80). Later, Argo *et al.* suggested that sustained histological response after short-term thiazolidinediones therapy for NASH is not lost, but it is related to sustained lifestyle modifications, especially physical activity (81). In contrast of promising results, meta-

analysis of 42 trials with rosiglitazone found that the drug was associated with significant increase in the risk of myocardial infarction (82). Possibly some additional insight will be provided by the ongoing US multicenter study (PIVENS) comparing pioglitazone with vitamin E or placebo (83).

LIPID-LOWERING AGENTS

Given the fact that hypertriglyceridemia and low HDL levels are defining elements of metabolic syndrome, and that those patients with elevated serum LDL-cholesterol and established coronary disease with other characteristics of the metabolic syndrome have the highest risk of major coronary events it was expected in course of time that studies exploring role of lipid-lowering agents in treatment of patients with NAFLD will be undertaken (84-86). A one year pilot study of clofibrate in the treatment of patients with hypertriglyceridemia and NASH, revealed no significant changes in levels of aminotransferases or liver histology (87). On the other hand results from a controlled trial of a four week treatment in patients with NASH, using gemfibrozil 600 mg/d showed significant biochemical improvements, but without histological confirmation (88). Three separate small pilot trails using HMG-CoA reductase inhibitor atorvastatin (10-80 mg/d) for 6-12 months have shown significant improvement or normalization in serum aminotransferase and lipids/cholesterol levels as well as improvement in hepatic inflammation in patients with NAFLD (89-91). Similar results were obtained by small pilot study using another HMG CoA reductase inhibitor pravastatin but without improvement in the fibrosis score (92). Greek open-label, randomised study in 186 non-diabetic patients with metabolic syndrome reported that combination treatment (atorvastatin+fenofibrate) combined with treatment of other components of metabolic syndrome (hypertension, impaired fasting glucose, and obesity) led to resolution of biochemical plus ultrasonographic evidence of NAFLD (93). A cumulative histopathological follow-up class study evaluated effect of statin therapy on histological outcome in patients with NAFLD (94). A significant reduction of liver steatosis at follow-up was noted in group of NAFLD patients taking statins. Recent animal study evaluated a novel combination therapy for NAFLD comprising ezetimibe, a cholesterol absorption inhibitor, and acarbose, an alpha-glucosidase inhibitor (95). Twenty four week treatment with combination therapy significantly reduced liver steatosis, inflammation and fibrosis, compared with long-term monotherapy with either drug. It also led to increase in the expression of microsomal triglyceride transfer protein and PPAR- α 1 in the liver, which may have led to the improvement in lipid metabolic disorder. One of the first questions that come in mind is potential hepatotoxicity of lipid-lowering agents especially in patients with underlying hepatic disease. There is enough evidence which show that standard statin therapy appears to be safe in patients with chronic liver disease with baseline elevations in aminotransferases with emphasis on use of a hydrophilic statin such as pravastatin (96-99). Recent studies have confirmed the safety of these agents in group of patients with NAFLD (100-101).

SUPPRESSING THE RENIN-ANGIOTENSIN SYSTEM

There is a certain role for angiotensin receptor blockers (ARB) in treatment of NAFLD patients. Renin-angiotensin system (RAS) has an important role in insulin resistance; suppressing the RAS improves intracellular insulin signaling through activation of PPAR-gamma, improves control of

adipokines production, and prevents hepatic stellate cell activation which consequently leads to prevention of hepatic inflammation and fibrogenesis (102). Therefore studies with losartan were performed in patients with NASH and arterial hypertension (103-105). Biochemical improvement with reduction in levels of blood markers of fibrosis, transforming growth factor- β 1 (TGF- β 1) and ferritin was reported. Also, after 48 weeks of treatment histological improvement was detected in majority of liver biopsy specimens. Because of these promising initial results further studies were performed in NAFLD patients. Enjoji *et al.* reported that telmisartan and olmesartan improve insulin sensitivity (HOMA-IR) and ALT levels and could be used as liver protecting agents in NAFLD patients (106). Romanian authors have compared efficacy of telmisartan *vs.* valsartan in 54 patients with NASH and mild-to-moderate hypertension, treated for 20 months (107). Telmisartan was superior according to the therapy induced mean HOMA-IR decrease per month, and reduction of NASH score. Recent animal study reported that 12 weeks of losartan treatment strongly reduces hepatic PAI-1 gene expression which could consequently lead to prevention of fatty liver disease (108). Another animal study gave us some more insights on the efficacy and mechanisms of action of telmisartan (109). It attenuates liver steatosis and fibrogenesis with decreased type I collagen and transforming growth factor-beta1 mRNA expressions and also suppresses the infiltration of macrophages into the liver, reduces the size of adipocyte in visceral fat tissue with an elevation of serum adiponectin concentration. Considering the standard and wide use of ARB in treatment of arterial hypertension, a component of metabolic syndrome, further studies in NAFLD patients should be encouraged.

URSODEOXYCHOLIC ACID

Ursodeoxycholic acid (UDCA), a hydrophilic bile acid, acts by competitive displacement of hepatotoxic hydrophobic endogenous bile acids that facilitate apoptosis, minimizing their toxicity and leading to a decrease in oxidative stress and hepatic injury (110). Several initial smaller studies have suggested potential benefit of UDCA treatment in patients with NASH alone or in combination with low-fat diet or lipid lowering agents (87, 90, 111-113). Improvements in liver enzymes levels, degree of liver steatosis and serum markers of fibrosis suggested a possible beneficial effect of UDCA in NASH patients. However, subsequent large randomized double-blind placebo-controlled trial involving 166 biopsy-proven NASH patients who were randomized to UDCA 13-15 mg/kg/day or placebo for 2 years did not show any significant difference in liver enzymes improvement or histology between the two study groups (114). Dufour *et al.* reported improved biochemistry and histology, mostly due to regression of hepatic steatosis, in biopsy-proven NASH patients treated with UDCA and vitamin E compared to UDCA alone or placebo (115). Recent study suggests that UDCA+vitamin E combination treatment also has metabolic effects, in addition to its beneficial cytoprotective properties (116). Improvement in terms of liver biochemistry and histology (of patients with NASH) was also followed by a decreased hepatocellular apoptosis and increased circulating levels of adiponectin.

VITAMIN E

Vitamin E (α -tocopherol) is an antioxidant that stabilizes biological membranes by protecting unsaturated fatty acids from lipid peroxidation and subsequent free radical reaction (110). A

relation between hepatic fibrosis and TGF- β 1 and pivotal role of TGF- β 1 in hepatic fibrogenesis and hepatocyte apoptosis has been reported (117-119). Vitamin E inhibits hepatic TGF- β 1 gene expression and subsequently attenuates cytokine stimulation of stellate cells and protects against liver fibrosis (120). Study by Hasegawa *et al.* with vitamin E administration (300 mg/day during 1 year) showed significant decrease in plasma TGF- β 1 levels along with normalization in liver enzymes and improvement in steatosis, inflammation and fibrosis on follow-up liver biopsy (121). Another small pilot study found that vitamin E administration normalizes liver tests (122). Subsequently, two small randomized controlled trials failed to provide any benefit of vitamin E (123, 124). A double blind, randomized, placebo-controlled trial that included 45 patients comparing vitamin E (1000 IU/day) and vitamin C (1000 mg/day) with placebo for 6 months showed no statistically significant difference in aminotransferase levels or degree of steatosis and inflammatory activity between vitamin E and placebo group, although an improvement in fibrosis score in vitamin E group was noted (125). In another study patients were randomized to vitamin E 600 IU/day plus ascorbic acid 500 mg/day (n=25) or placebo (n=28), and treated for 24 months (126). All patients also underwent lifestyle intervention (diet and increased physical activity). Although significant improvement occurred in the levels of aminotransferases along with grade of steatosis, lobular inflammation, hepatocyte ballooning, and NAFLD activity score, there was no significant difference in all these parameters between the two groups. Apparently, vitamin E plus ascorbic acid does not seem to increase the efficacy of lifestyle intervention alone. In recent animal study, vitamin E showed an antioxidant effect on the liver but it was not sufficient to improve liver histology (127). This suggests that vitamin E alone, characterized only with antioxidant properties, is insufficient to inhibit progression of NAFLD and combination with other therapeutic modalities are needed. A multicentered, randomized, placebo-controlled, double-blinded clinical trial comparing metformin with vitamin E or placebo in 173 nondiabetic children with histologically confirmed NAFLD will hopefully give more information about their effect on liver histology (128).

POLYUNSATURATED FATTY ACIDS

Polyunsaturated fatty acids (PUFA) are ligands of peroxisome proliferator-activated receptor α (129). Deficiency of the n-3 series long-chain polyunsaturated fatty acids (LCPUFA) and subsequent increased n-6/n-3 fatty acid ratio leads to impairment of PPAR- α activity in the liver followed by higher hepatic uptake of free fatty acids, a decrease of hepatocyte microsomal, peroxisomal and mitochondrial β -oxidation, a reduced synthesis of fatty acid-transport proteins and an up-regulation of SREBP-1 (130-132). Studies with animals showed that diet intake enriched with n-3 PUFA increased insulin sensitivity, reduced liver fat accumulation and improvement of steatohepatitis (132-134). Araya *et al.* reported deficiency in the n-3 series long-chain polyunsaturated fatty acids (LCPUFA) and subsequent increased n-6/n-3 fatty acid ratio in NAFLD patients when compared with controls (130). It was suggested that depletion of LCPUFA may be the result of defective desaturation of PUFA due to inadequate intake of precursors and increased peroxidation of LCPUFA. In the first non-randomized study in humans, 42 NAFLD patients treated with n-3 PUFA (1 g/day) for 12 months showed reduction of ALT serum levels and triglycerids, regression in echotexture, and increased doppler perfusion index (136). Improvement in ALT, serum lipid levels and normalization of ultrasonographic

features were reported in a larger trial of 144 patients with NAFLD treated with n-3 PUFA (2 g/day) for 24 weeks (137). Disadvantage of these studies is the lack of liver histology and ultrasound scoring system description.

OTHER AGENTS

As it is postulated, adipokines and cytokines such as adiponectin, TNF-alpha (TNF- α) and IL-6 have an important role in pathogenesis and severity of NAFLD (7). Studies with agents which target adipokines are therefore a logical approach in search for new NAFLD therapies, but up until recently only a few drugs have been evaluated. Pentoxifylline has recently emerged as point of new interest because of its nonspecific TNF-alpha inhibitor property. First two studies reported significant biochemical improvement in ALT levels and HOMA-IR in NASH patients treated with pentoxifylline (400 mg t.i.d.) for 6 to 12 months, while results from a third study confirmed correlation between biochemical and histological improvement (reduction in steatosis, lobular inflammation and fibrosis stage) after 12 month treatment (138-140). On the other hand another study (by Lee YM *et al.*) did not find any significant reductions in ALT levels in placebo-controlled trial of NAFLD patients treated with pentoxifylline in combination with exercise and diet for 3 months (141). Infliximab is a chimeric monoclonal antibody that selectively and potently blocks TNF- α , while tocilizumab is a humanized IL-6 receptor antibody. Future human trials using these agents in patients with NASH could be interesting. Resveratrol is a polyphenol with anti-inflammatory effect mediated partly through a decrease in TNF- α production (142). A study using animal model of steatosis reported that hepatic steatosis and ALT levels were significantly decreased in rats treated with resveratrol (143). Hyperuricemia is a marker for cardiovascular risk factors such as hypertension, dyslipidemia and diabetes. Allopurinol, which inhibits the generation of uric acid, has recently become interesting because of its potential role in treatment of metabolic syndrome and NAFLD (144-146). Probiotics have been used in small studies of patients with NASH, because of their potential immunoregulatory and anti-inflammatory activity (147). Loguericio *et al.* reported some beneficial effect of VSL#3 probiotic on ALT levels as well as on other markers of lipid peroxidation in 22 NAFLD patients (148). Velayudham *et al.* reported that VSL#3 can affect liver fibrosis through modulation of collagen expression and impaired TGF- β signaling (149). Ding *et al.* have shown that administration of exendin-4, glucose-like protein-1 (GLP-1) receptor agonist, induces regression of hepatic steatosis in ob/ob mice by improving insulin sensitivity. Although promising results in decreasing serum aminotransferase level and improving hepatic steatosis on animal models have been perceived, clinical trials are still missing (150, 151).

CONCLUSION

According to the recent surveys, the traits of metabolic syndrome can be found in up to one-fourth to one-third of general population. It is therefore not surprising that NAFLD has become the most common chronic liver disease in Western countries, owing its rise primarily to the increasing prevalence of obesity in virtually all age groups. We are still pretty much unable to make a definitive diagnosis of NAFLD or to distinguish between NAFLD and NASH without liver biopsy, although recent advances through research of single nucleotide polymorphisms and development of noninvasive markers of hepatic fibrosis could improve the situation in the near future.

NAFLD is a "silent disease" with slowly progressive nature which requires long-term follow-up. Most of the patients can be safely managed conservatively with a repeated check of liver tests without any need for further invasive work-up or medical treatment. Standard approach to these patients should be an effort to control and treat the potential risk factors such as obesity, hyperlipidemia, hypertension and diabetes. Weight reduction has clearly been associated with improvement in liver biochemical tests and prevention of further hepatic injury. It should be gradual, since rapid weight loss can aggravate underlying liver disease. Over the last two decades large numbers of pharmacologic agents have been studied as potential treatment options for NAFLD. However, the majority of studies have small sample sizes, short follow-up duration, and hence also limited statistical power, which makes it rather difficult to draw any definitive conclusions about the use of these agents. Only a few of described agents have been shown to have substantial effects and until today no single or combination therapy has come upfront in a way that it could be firmly recommended in the treatment of NAFLD. High prevalence and natural history potentially leading to end-stage liver disease clearly indicate the necessity for efficient therapy for a large number of these patients. We believe that firmer conclusions about NAFLD treatment will become available in the near future, mainly through diverse ongoing and forthcoming studies which address not only the potential treatment options, but also disease pathogenesis and possibility of categorization of patients with respect to disease prognosis.

Conflict of interests: None declared.

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Received: October 15, 2009

Accepted: December 15, 2009

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