An eBook on Type2 Diabetes

Chapter 1

Nonalcoholic Fatty Liver Disease in Diabetes: From Diagnosis to Treatment

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Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase ; CT: Computed tomography; DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; HCC: Hepatocellular carcinoma; IR: Insulin resistance; MRI: Magnetic resonance imaging; MRS: Magnetic Resonance Spectroscopy; NAFLD: Non-alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; SGLT-2: Sodium-glucose co-transporter 2; T2DM: Type 2 diabetes mellitus; TE; Transient elastography; TZDs: Thiazolidinediones; γ-GTP: γ-glutamyl transpeptidase

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated liver enzymes and chronic liver disease, affecting approximately 30% of the general population [1]. NAFLD involves the presence of hepatic steatosis not caused by alcohol intake. In NAFLD, the histological examination of liver biopsy reveals excess accumulation of lipids (representing predominantly triglycerides) within hepatocytes. NAFLD may progress from steatosis to steatohepatitis, cirrhosis, and finally liver failure [1].

Obesity is an established risk factor for type 2 diabetes mellitus (T2DM), cardiovascular diseases, gall-bladder disease, osteoarthritis and NAFLD. It is noteworthy that up to 70% of patients with T2D develop NAFLD [2] and both NAFLD and T2DM share common pathogenic mechanisms as well as complications. In addition, the prognosis for patients with NAFLD and T2DM is worsened due to increased risk for cardiovascular disease and hepatocellular carcinoma (HCC) [2]. Patients with NAFLD are at increased risk for cardiovascular disease since NAFLD promotes dyslipidemia [3], hyperinsulinemia [4] and subclinical inflammation [5], all of which are potentially atherogenic risk factors. Furthermore, NAFLD increases the risk for HCC. A study reported that between 2004 and 2009, HCC related to NAFLD and its more aggressive form, nonalcoholic steatohepatitis (NASH), increased by 9% [6].

2. Epidemiology

Due to the obesity epidemic, NAFLD has become the leading cause of chronic liver disease in the United States, with an estimated prevalence of 34% in the general population [7]. In patients with T2DM, the prevalence of NAFLD ranges from 57% to 80%, depending on the diagnostic test [8-10]. In a recent study in obese patients with T2DM and normal liver aminotransferases, the prevalence of NAFLD was 56% while more than half of those undergoing a liver biopsy had NASH [11].

Presence of T2DM has been associated with a faster progression to NASH and advanced fibrosis. In a study in T2DM patients [12], the prevalence of NASH was 78% while about 50% of study patients had advanced fibrosis. Another study in T2DM patients [13] showed the independent association between T2DM with NASH. In a report from the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) [14], patients with NASH were much more likely to have T2DM than those with milder liver disease. At last, two recent large population-based studies using noninvasive imaging tools have confirmed that 17% of patients with T2DM have significant fibrosis [15,16].

3. Pathogenesis of NAFLD

The pathophysiology of NAFLD is complex and multifactorial. There are well known relationships between NAFLD, obesity [2] and T2DM independent of obesity [17]. It is well recognized that insulin resistance (IR) is a major factor in the pathogenesis of NAFLD [18]. IR is characterized by increased plasma levels of tumor necrosis factor-a, interleukin-6 [19] and plasma free fatty acid levels. The above inflammatory factors in combination with fasting and postprandial hyperglycemia result to hepatic steatosis [20]. In the setting of IR, lipolysis in the adipose tissue is increased, resulting in an increased release of FFAs into the circulation that, in turn, results in lipotoxicity, lipid accumulation in liver and skeletal muscle, leading to cellular dysfunction and/or death [21]. Additionally, impaired very low-density lipoprotein secretion, which commonly occurs with IR, further contributes to hepatic fat accumulation. In addition to increased production of FFA, NAFLD results in increased hepatic gluconeogenesis that causes worsening of hyperglycemia and IR.

T2DM and IR have been linked to worsening of liver damage and hepatocyte apoptosis [20,22]. The progression to NASH is heralded by the oxidative stress that results in lipid peroxidation, release of pro-inflammatory cytokines that then result in fibrosis and inflammation. Epidemiologic data suggest that T2D patients have a higher prevalence of NASH and are more likely to develop advanced fibrosis [23], and a twofold higher risk of progression to cirrhosis and HCC, as well as higher risk of mortality from cirrhosis or HCC [24-26].

Several genes have been found to be associated with an increased risk of NAFLD/

NASH, including those associated with glucose metabolism, IR, hepatic lipid metabolism, and oxidative stress and inflammation [27,28]. Furthermore, recent studies revealed the role of gut microbiota in the development of obesity, T2DM, and NAFLD. Patients with NAFLD have an adverse intestinal microbial composition that predisposes to fatty liver, as well as increased intestinal permeability, that is associated with progression to NASH [29,30].

4. Diagnostic Evaluation

For the diagnosis of NAFLD, there should be no history of alcohol consumption (defined as an intake of greater than 20 g/day), no exposure to steatogenic medications, hereditary and metabolic liver diseases, nutritional imbalance pregnancy and chronic hepatitis C [31]. Abnormal liver functions are unusual in the majority of cases of NAFLD. However, only 20% of the patients with NAFLD exhibit mild elevation of aminotransferases [32]. The usually seen abnormalities are the elevation of γ-glutamyl transpeptidase (γ-GTP) and alanine aminotransferase (ALT) while the ratio between aspartate aminotransferase (AST) and ALT has been used to identify patients with advanced fibrosis and a value > 1 may predict advanced fibrosis in patients with NAFLD [33].

Ultrasound (US) is the common diagnostic approach for the diagnosis of NAFLD since it is a safe, inexpensive and readily available method. US has high specificity and sensitivity for steatosis, >30%; however, it cannot accurately detect steatosis between 5 and 30%, and in very obese patients, its sensitivity is reduced [34]. US of the liver has a reported sensitivity of 91 %, specificity of 93 %, positive predictive value of 89 % and a negative predictive value of 94 %, for patients with at least 30 % steatosis [34-38].

Computed tomography (CT) allows quantitative and qualitative evaluation of liver steatosis with a higher accuracy [39, 40]. CT scan has a sensitivity of 73–100% and a specificity of 95–100% for diagnosis of NAFLD [41] but involves exposure to ionizing radiation. Magnetic resonance imaging (MRI) is reported to detect liver fat content as low as 3% [42]. Magnetic Resonance Spectroscopy (MRS) is one of the most accurate methods for the evaluation of liver steatosis, has a strong correlation with histology and can detect very low levels of steatosis [43].

Transient elastography (TE) is used to diagnose liver fibrosis noninvasively. This is an US-based technique to measure elasticity or stiffness in hepatic parenchyma via an ultrasonic probe, either as an in-patient or as an out-patient procedure. TE has a high sensitivity for the diagnosis of varying degrees of liver fibrosis and cirrhosis [44]. However, its application and precision in obese patients are debated because its accuracy in predicting cirrhosis is influenced by the necroinflammatory activity and presence of fat in the liver [43,44].

Given the limits of liver biopsy and the cost of some imaging techniques, noninvasive

scoring tools have been developed and validated based on serum biomarkers and/or clinical parameters, such as age, gender, body mass index, waist circumference, and the presence of metabolic syndrome, T2DM, and/or IR [44]. However, liver biopsy is the gold standard diagnostic test for the diagnosis of NAFLD and NASH. The histological diagnosis of NAFLD is defined as the presence of lipid deposit in more than 5% of the hepatocytes independent of the localization into the hepatic lobule. It helps to assess the severity and prognosis of disease. However, it is invasive, costly, associated with sampling errors, bleeding risk and even death [45].

5. Management of NAFLD in patients with T2DM

5.1 Lifestyle modification

Lifestyle modification and weight loss is the corn stone of the management of NAFLD. The usual goal in obese subjects is to achieve sustained weight loss of 7-10% of body weight with a combination of a balanced, calorie-restricted diet and increased physical activity. A rate of weight loss of up to 1 kg/week is considered to be safe in the setting of NAFLD.

Many studies indicated that diet and exercise ameliorate aminotransferases and steatosis, as evaluated by imaging techniques in patients with NAFLD. Even a modest weight reduction of 7% was associated with significant improvements in steatosis, lobular inflammation, ballooning and NAFLD Activity score in another recent study [46]. A dietary intervention for 2 weeks [47] resulted in a body weight reduction of 4.3% and a further reduction in hepatic triglyceride content by 42% in subjects with NAFLD. Lifestyle modification following the Mediterranean diet for 6 weeks was associated with significant reduction of hepatic steatosis as assessed by MRS with improved insulin sensitivity in nondiabetic NAFLD patients [48]. A meta-analysis of 28 randomized clinical trials demonstrated that physical exercise significantly reduced intrahepatic lipids and markers of hepatocellular injury [49]. Furthermore, in patients with NASH a weight loss of 10% improves steatosis, necrosis, and inflammation on liver biopsies, [46,50] a finding that is even more consistent after bariatric surgery [51,52]. A study in patients with NAFLD and T2DM showed that sleeve gastrectomy might have better effects on NAFLD than gastric bypass, while the effects on blood glucose are similar [53].

5.2 Metformin

Metformin improves insulin sensitivity by several mechanisms including decreasing gluconeogenesis in the liver, increasing glucose uptake in the periphery, and increasing fatty acid oxidation [54]. Although metformin would theoretically improve NAFLD, a recent clinical trial did not show any significant benefit of metformin treatment [54]. In accordance, a meta-analysis of nine studies involving 417 participants on the use of metformin dosed at 0.5–3 g/day for NAFLD [55] showed no histological response to therapy, including steatosis,

inflammation, hepatocellular ballooning, and fibrosis. Another study, using liver biopsies, in patients with NAFLD who took metformin (20 mg/kg/day in three divided doses) for 48 weeks [56], showed that only three patients had a reduction in steatosis at the study's end.

An important benefit of metformin therapy is its contribution to weight loss, possibly through its impact on IR [57]. A study showed that metformin therapy showed a positive correlation between weight loss and improvements in hepatocellular injury and inflammation as well as a significant reduction in homeostatic model assessment for IR [58]. Furthermore, improvements in ALT and AST, a common laboratory finding in NAFLD, have been seen with metformin treatment in almost all patients in the above studies [59].

5.3 Sulfonylurea

Until now, role of sulfonylurea in NAFLD with T2DM has not been established. However, retrospective data have showed that sulfonylurea treatment in T2DM patients with NAFLD increases the risk of fibrosis due to the profibrotic effect of insulin [60]. However no adjustment was made for glycemic control or diabetes duration. In a combined analysis of studies comparing oral hypoglycemic agents taken for one year, gliclazide treatment was associated with a modest deterioration of liver enzymes when used either as a single agent or in combination [61].

5.4 Thiazolinediones: pioglitazone

Thiazolidinediones (TZDs) are ligands that target the transcription factor peroxisome proliferator activated receptor (PPAR)-γ, with broad effects on glucose/lipid metabolism through modulation of substrate supply, insulin signaling and mitochondrial fatty acid oxidation [62].

The first randomized controlled study of pioglitazone in patients with NASH included patients with prediabetes and T2DM and lasted 6-month. Pioglitazone treatment significantly ameliorated hepatic steatosis and necroinflammation while there was evidence that fibrosis could be reversed in these patients. [63] This study was important in establishing that NASH could be reversed within a relatively short period of time (6 months). Recently, [64] a 36-month study in 101 patients with prediabetes or T2DM and NASH showed histological and metabolic benefit with long-term pioglitazone treatment. Treatment was well tolerated, with only mild weight gain (2.5 kg versus placebo).

Potential side effects associated with TZDs include weight gain, development of shortness of breath or congestive heart failure and a mild bone loss in women [65]. A possible association between pioglitazone and bladder cancer was recently revealed, but a 10-year prospective study showed no association between pioglitazone and bladder cancer [66].

5.5 Incretins

5.5.1 Dipeptidyl peptidase-4 (DPP-4) inhibitors

DPP-4 inhibitors mechanism of action is the blockade of the protein DPP-4 that degrades glucagonlike peptide-1 (GLP-1) and regulates postprandial glucose homeostasis [67, 68]. Studies with DPP-4 inhibitors in patients with NAFLD have reported mixed results. In animal models of NASH, DPP-4 inhibitors prevented the development of steatohepatitis by affecting both inflammatory and fibrosis pathways (reduced expression of proinflammatory mediators, attenuation of endoplasmic reticulum stress, reduction in hepatocyte apoptosis, decreased accumulation of fibronectin and alpha-smooth muscle actin, and reduction in plasminogen activator inhibitor 1 expression) [69,70].

In humans results for sitagliptin have been mixed too. In Japanese patients with T2DM and NAFLD reduction in plasma aminotransferases have been reported with sitagliptin [71, 72]. Four months of treatment with sitagliptin 50 mg/day in 30 T2DM patients with NAFLD was associated with significant decreases in AST, ALT and γ-GTP levels [72]. In another study of 15 patients [45], one year sitagliptin treatment was associated with significant reduction in NASH scores and a trend towards improved hepatic steatosis. Significant reductions in AST and ALT levels and body mass index were also observed [73]. In contrast, in another study plasma aminotransferases did not improve in patients with biopsy-proven NAFLD treated with sitagliptin for 12 months [74]. In a 24-week study in overweight patients with T2DM, sitagliptin was compared with liraglutide and placebo for their impact on hepatic fat content and hepatic fibrosis. At the end of the study no difference in hepatic fat content and liver fibrosis was observed between the three groups [75]. Vildagliptin treatment for 6 months T2DM patients with mild steatosis [76] showed a reduction in hepatic triacylglycerol accumulation. In this study, there was no improvement in liver, muscle or adipose tissue insulin sensitivity. Another studied DPP-4 inhibitors is alogliptin, which showed a decrease in NASH, ferritin, insulin, type 4 collagen 7S score in NAFLD patients with T2DM followed for 12 months [77].

In summary, current evidence suggests that DPP-4 inhibitors do not improve histologic features of NAFLD. However, further randomized, placebo-controlled trials of larger sample size over longer follow-up periods are needed to assess the role of DPP-4 inhibitors in patients with T2DM and NAFLD.

5.5.2 Glucagon-like peptide-1 (GLP-1) receptor agonists

This class of drugs acts on the pancreas, brain, and adipose tissue in a way similar to physiological GLP-1 and exerts its antidiabetic effect through controlling food intake, energy absorption, and glucose-dependent insulin secretion [78]. A number of animal studies have shown that GLP-1 analogs improve hepatic insulin sensitivity and decrease steatosis [79,80],

and even fibrosis [81]. Exendin-4 significantly reduces hepatic de novo lipogenesis in vitro and in vivo [82]. In a study in 60 newly diagnosed T2DM patients with obesity, NAFLD with elevated liver enzymes, exenatide plus insulin glargine was compared to insulin aspart plus insulin glargine. Levels of ALT, AST, and γ-GTP in the exenatide group were significantly lower than in the intensive insulin group, and the reversal rate of fatty liver was significantly higher in the exenatide group [83]. Meta-analysis of studies with lixisenatide versus placebo or active comparators showed that lixisenatide increased the proportion of patients with normalization of ALT compared with placebo or active comparators [84].

Liraglutide reduced liver fat content as assessed by MRS in patients with uncontrolled T2DM thanks to its weight-lowering effect, whereas insulin glargine, despite its effective glycemic control, produced no improvement in weight loss and liver fat content [85]. However, another study that compared the effect of a 12-week course of insulin glargine versus liraglutide among 35 patients with T2DM inadequately controlled on metformin monotherapy or in combination with other oral antidiabetic drugs showed that, despite the similar glycemic control, the insulin group showed significant reduction in liver fat burden assessed radiologically, whereas no significant change was detected in the liraglutide group [86]. In agreement with the above results, no changes in liver fat content and surrogate biomarkers of fibrosis were shown in a study comparing the effect of a 12-week course with liraglutide versus sitagliptin or placebo among 52 overweight T2DM patients on metformin or sulfonylurea [75]. A significant decrease in plasma aminotransferase levels and hepatic steatosis was observed with liraglutide treatment at the dose of 1.8 mg in a meta-analysis using six 26-week, phase III, randomized controlled T2DM trials from the LEAD (Liraglutide Efficacy and Action in Diabetes) programme [87]. This effect was likely mediated by liraglutide's weight loss and glycemic control effects.

In the Liraglutide Efficacy and Action in NASH (LEAN) study, which was a randomized, placebo-controlled trial consisting of 52 patients with biopsy-proven NASH, liraglutide (1.8 mg daily) for 48 weeks was associated with greater resolution of steatohepatitis and less progression of fibrosis. Among the LEAN study population, 32.7% of participants were patients with T2DM. Patients treated with liraglutide had a significant reduction of body weight, fasting plasma glucose and A1c levels [88].

Dulaglutide, a long-acting GLP-1R agonist, has been approved at once weekly doses of 0.75 mg and 1.5 mg. A recent study showed the favorable effects of once weekly dulaglutide for 12 weeks on body weight, HbA1c and ALT levels in patients with biopsy-proven NAFLD, although some of them unfortunately discontinued this agent by 12 weeks due to gastrointestinal disorders [89].

However, additional placebo-controlled or head to head trials are required to investigate these newer agents.

5.6 Sodium-glucose co-transporter 2 (SGLT-2) inhibitors

These agents inhibit the reabsorption of glucose in the proximal tubular system with a marked reduction of plasma glucose levels [90,91]. Their use is also associated with a reduction of total body weight, possibly as a secondary effect of caloric loss and increased diuresis [92]. SGLT-2 inhibitors treatment showed antifibrotic properties in animal models of NASH [93]. In T2DM patients, levels of liver enzymes were decreased by SGLT-2 inhibitors treatment [94]. Recently, meta-analysis of studies with canagliflozin versus placebo and active comparator (sitagliptin) showed significant reductions in plasma ALT with canagliflozin 300 mg [95]. Changes in the ratio AST/ALT were explained by the reduction in HbA1c and body weight caused by canagliflozin.

Many small studies have reported that dapagliflozin treatment had beneficial effects on levels of liver enzymes, liver fat content, and/or liver stiffness in patients with T2DM and NAFLD [96]. The Effects of Omega-3 Carboxylic Acids and Dapagliflozin on Liver Fat Content in Diabetic Patients (EFFECT-II) study, showed that combined treatment with dapagliflozin and omega-3 carboxylic acids significantly reduced liver fat content as assessed by MRI [97]. Another 6 months study in 55 Japanese T2DM patients showed that dapagliflozin treatment significantly reduced liver fat accumulation as assessed by the liver-to-spleen attenuation ratio using abdominal CT compared with non-SGLT-2 inhibitor treatment [98]. Finally, In the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus (EMPA-REG OUTCOME) trial, empagliflozin reduced aminotransferase levels in T2DM patients [99]. The Effect of Empagliflozin on Liver Fat Content in Patients With Type 2 Diabetes (E-LIFT) trial, showed that empagliflozin significantly reduced liver fat as measured by MRI [100].

However, since SGLT-2 inhibitors is a new class of antidiabetic agents, further studies are needed to define its role in T2DM patients with NAFLD.

5.7 Insulin

Since NAFLD is characterized by significant IR, high doses of insulin are frequently needed, particularly in morbid obese subjects. A 12 weeks study in T2DM patients inadequately controlled on oral antidiabetic drugs showed that insulin glargine therapy had a significant effect on hepatic fat reduction [86]. Paradoxical, while insulin enhances lipogenesis in vitro, [101] human studies show improvement in liver fat, which could be attributed to improved hepatic insulin sensitivity, and reduced gluconeogenesis [102,103].

6. Conclusion

The worldwide prevalence of NAFLD will continue to rise because of the alarming rise in obesity globally. Diagnosis of NAFLD depends on the presence of more than 5% fat in the

liver by radiological imaging or hepatic biopsy. Pathogenesis of NAFLD is complex although the accumulation of intrahepatic lipid is central while IR is one of the main pathogenetic mechanisms leading to NAFLD. Management of T2DM patients with NAFLD is based primarily on lifestyle modifications (diet and exercise), while antidiabetic agents have showed promising results. It must be noticed that a new era in the management of NAFLD with T2DM with novel anti-diabetic agents will dramatically change the management of the disease. Among them, GLP-1R agonists and SGLT2 inhibitors may be the most promising agents for T2DM patients with NAFLD, because of their effects on HbA1c decrease and weight reduction. However, there are many challenges in the diagnosis and management of NAFLD in patients with T2DM.

7. References

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