Non-alcoholic Fatty Liver in the Pathogenesis of Diabetes

15

Cristiane A. Villela Nogueira and Nathalie Carvalho Leite

Introduction

Non-alcoholic fatty liver disease [1] is prevalent worldwide and is recently one of the leading causes of chronic liver disease in the occident due to obesity-related epidemic and metabolic syndrome [2]. NAFLD presents with different phenotypes and may progress to cirrhosis and hepatocellular carcinoma. Moreover, it may be the leading cause for liver transplant in the next decade [3]. NAFLD was formerly identified in 1980, when Ludwig et al. described a small series of patients with liver histology characterized by fat accumulation, hepatic necroinflammation, and, in most cases, fibrosis, in the absence of a history of excessive alcohol consumption [4].

Definition of NAFLD

Currently, NAFLD is defined as the presence of macrovesicular steatosis in ≥5% of hepatocytes in individuals who consume little or no alcohol. NAFLD is divided into two major subtypes that comprise different phenotypes histologically identified: non-alcoholic fatty liver (NAFL, also termed simple steatosis), the non-progressive form of NAFLD that rarely develops into cirrhosis, and NASH, the progressive form of NAFLD that can lead to cirrhosis and hepatocellular carcinoma [1] and is associated with an increase of liver-related mortality. NASH is characterized by the presence of steatosis, ballooning degeneration, and lobular inflammation, with or without peri-sinusoidal fibrosis on liver histology [5].

C. A. Villela Nogueira (✉)
School of Medicine, Internal Medicine Department, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

N. C. Leite
Clementino Fraga Filho University Hospital, Internal Medicine Division, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Epidemiology

The background for the high prevalence of NAFLD is multifactorial, being related to sedentarism, Western lifestyle worldwide, obesity, as well as to genetic factors. It is present in almost 30% of the general population [6]. The prevalence of NAFLD in Europe and the Middle East ranges from 20% to 30% [7]. In the USA, one-third of the population is now obese, and one-third of American adults are thought to have NAFLD (Centers for Disease Control and Prevention *Overweight and Obesity* [online], http://www.cdc.gov/obesity/data/adult.html (2012). NAFLD prevalence in Japan and China is similar to that in Europe (20–30% in Japan and 15–30% in China, respectively) [8]. In the Indian subcontinent, the prevalence of NAFLD in urban populations ranges from 16% to 32%; however, in rural India, where most people have traditional diets and lifestyles, the prevalence is around 9%, lower than in urban population [9]. In Latin America, the prevalence of NAFLD has been reported to range from 17% to 33% [10]. Data is lacking in the African continent; however, one study from Nigeria, which included patients with and without diabetes mellitus, identified a prevalence of 9.7% [11]. Regarding pediatric population, the prevalence of NAFLD varies from 3% to 10%, rising up to 40–70% among obese children [12].

Patients with NAFLD and metabolic syndrome share the same risk factors: obesity, type 2 diabetes mellitus, dyslipidemia, and insulin resistance. Diabetes has a huge impact not only on its prevalence worldwide but also on NAFLD severity [13]. The prevalence of ultrasonographic NAFLD in diabetic patients may be as high as 70% [13, 14].

Although cardiovascular death is the most common mortality-related factor among NAFDL population, increasing data regarding liver-related death due to liver dysfunction and hepatocellular carcinoma [1] has been increasingly reported. Although HCC is usually diagnosed in patients with NAFLD-related cirrhosis, it has also been detected in non-cirrhotic NAFLD. However, its true incidence and risk is still unknown [1]. Compared to viral

J. Rodriguez-Saldana (ed.), *The Diabetes Textbook*, https://doi.org/10.1007/978-3-030-11815-0_15

hepatitis, the progression of liver fibrosis in NAFLD seems to be slower (patients developing cirrhosis 28–57 years) [15]; however, the burden of patients with NAFLD is higher than those with hepatitis C [16]. At present, NASH cirrhosis is the third leading indication for liver transplantation in the USA [17]. In the forthcoming decades, due to a projected increase in HCC incidence, a change in the burden of related cases of HCC is expected, moving from viral hepatitis to NASH-related cirrhosis as the major risk factor for HCC worldwide [7].

Clinical Manifestations

The clinical presentation of NAFLD is insidious. Most patients are generally asymptomatic at diagnosis and are often referred from an internist with an ultrasound that demonstrates liver steatosis. Indeed, abdominal ultrasonography, owing to its non-invasive profile and easy accessibility, is the main screening and diagnostic method for NAFLD [18], although it is limited for patients that have more than 33% steatosis on liver biopsy. Patients who are symptomatic usually have non-specific symptoms like fatigue and a dull pain or heaviness in the right hypochondria. However, a physical exam with signs of insulin resistance like acanthosis nigricans, an enlarged waist circumference (usually over 88 cm in women and 102 cm in men), and overweight should also be a clinical clue to the diagnosis of NAFLD [19]. It's also important to be aware of some clinical conditions that may be associated with insulin resistance like polycystic ovarian syndrome in young women, which usually presents with obesity, hirsutism, acanthosis, and other diseases like hypothyroidism and sleep apnea disease which are closely related to an increased prevalence of NAFLD (Bano, 2016 #990) [20].

Diagnosis

Most patients with NAFLD are diagnosed by incidental elevated liver enzymes or imaging studies suggesting hepatic steatosis [21]. When NAFLD is suspected, the first step to confirm its diagnosis is to exclude other known etiologies of chronic liver diseases like drug-related steatosis [22, 23], viruses [24], and alcohol. As previously described, a careful history of alcohol ingestion and medications that are related to steatosis must be taken. Of note, some NAFLD patients with excessive alcohol intake may have both alcoholic and non-alcoholic fatty liver disease [25]. The average amount of alcohol that is allowed for patients with NAFLD have been under debate, but so far, although small to moderate amounts of alcohol might be related to a decrease in cardiovascular risk, patients with NAFLD should refrain from drinking alcohol [26]. Generally, for the diagnosis of NAFLD, the upper limit for alcohol intake would be a maximum of 30 g alcohol/day.

The different phenotypes of NAFLD are simple steatosis, steatohepatitis, and fibrosis. However, so far, due to the lack of specific and accurate biomarkers, only liver biopsy can accurately identify steatohepatitis. Steatosis is the most prevalent phenotype, and patients with simple steatosis have a benign course of the disease. Although NAFLD is the most common diagnosis in patients with incidental abnormal liver function tests [27], laboratorial tests are of minor value since most of patients with NAFLD including those with more advanced disease may present normal inflammatory liver enzymes [28]. Another drawback in laboratorial diagnosis of NAFLD is that as fibrosis progresses, both inflammatory enzymes such as AST and ALT and steatosis decrease. On the other hand, patients with persistent abnormal liver enzymes are those who usually present NASH on liver biopsy as well as other liver comorbidities like viral or autoimmune hepatitis. In conclusion, routine AST/ALT do not differentiate steatosis, NASH, or the stage of fibrosis [29].

Liver Biopsy and Non-invasive Markers of Fibrosis

As already stated, the only way to accurately diagnose the different phenotypes of NAFLD is through a liver biopsy. This is an invasive method prone to inter-observer and intra-observer disagreement. In addition, it is painful and difficult to be performed in such a high burden and widespread disease. Due to these drawbacks, the search for non-invasive methods to identify different spectrum of the disease is currently under research. So far, steatosis and fibrosis can be identified by non-invasive methods that vary from serological scores to image methods, and since the presence of fibrosis is the most important prognostic marker of the disease, it is reasonable to develop non-invasive methods that correctly identify or exclude liver fibrosis. At present there are a great number of serological scores that can be used to assess patients with NAFLD. They are usually applied as screening tools to identify patients with higher risk to the progressive forms of NAFLD. The most commonly used is the NAFLD fibrosis score ($1.675 + 0.037 \times$ age $+ 0.094 \times$ BMI $+ 1.13 \times$ IFG/Diabetes $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelets $- 0.66 \times$ albumin), where a score <-1.455 excludes fibrosis (NPV 88–93%) and >0.676 predicts fibrosis (PPV 82–90%) and FIB-4 [[30]/(Platelets* Sqrt (ALT)] which has been defined as a useful score to predict fibrosis in NAFLD patients as well: a result <1.35 excludes fibrosis (NPV 95%) and >3.25 predicts fibrosis (PPV~ 70%). McPherson et al. compared the performance of simple serological tests to predict fibrosis, and the results are shown in Table 15.1 [31].

Table 15.1 Diagnostic performance of serologic scores to evaluate fibrosis in NAFLD

Test	AUC	Cutoff	Sens (%)	Spec (%)	PPV (%)	NPV (%)
NAFLD fibrosis score	0.81 (0.71–0.91)	−1.45 0.676	78 33	58 98	30 79	92 86
FIB-4	0.86 (0.78–0.94)	1.30 3.25	85 26	65 98	36 75	95 85
BARD	0.77 (0.68–0.87)	2	89	44	27	95
APRI	0.67 (0.54–0.8)	1	27	89	37	84
AST/ALT ratio	0.83 (0.74–0.91)	0.8 1	74 52	78 90	44 55	93 89

Legend: *NAFLD* non-alcoholic fatty liver disease, *BMI* body mass index, *IFG* intolerant fasting glucose, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *NPV* negative predictive value, *PPV* positive predictive value

NASH is the phenotype of NAFLD that points to a progressive form of the disease, and so far, only liver biopsy is able to make this diagnosis. The histological definition of NASH comprises the triad of steatosis, cell injury [32], or any amount of lobular or portal inflammation. Of note, fibrosis is not required for the diagnosis of NASH. Semiquantitative histological scoring systems have been proposed for NAFLD, but they are not useful in clinical practice, and each has certain limitations. Recently, Bedossa et al. developed a new histological classification for NAFLD: the FLIP algorithm and the SAF (steatosis, activity, fibrosis) which assesses separately the grade of steatosis [32], the grade of activity [32], and the stage of fibrosis [32]. This algorithm and score may improve the agreement between pathologists when describing fibrosis stage [32].

Other non-invasive tools that have been useful as screening methods for the identification of patients with higher risk of fibrosis are transient elastography (TE) [33], two-dimensional shear wave elastography (2D-SWE), acoustic radiation force impulse (ARFI) which is a type of point shear wave elastography, and elastorressonance. Although elastoressonance has been considered the most accurate method for the identification of liver fibrosis, its use is limited as a screening method by cost and accessibility.

TE uses an ultrasound displacement M-mode and A-mode image produced by the system. It has two probes, M and XL. The XL probe was designed for obese patients, which has increased the success rate of the exam in patients with NAFLD since most are obese and, before the development of the XL probe, most exams were unreliable. Currently, all patients with a skin-to-liver capsule distance (SCD) of >25 mm should be assessed with the XL probe. Measures obtained with the XL probe are generally 1.5 kPA lower than those achieved with the M probe [34]. TE results under 7.9 kPa have a high negative predictive value for advanced fibrosis (97%) and should be employed in daily practice to decide about performing a liver biopsy in patients with NAFLD [35].

Two-dimensional shear wave elastography (2D-SWE) evaluation needs to be performed in a well-visualized area of the right liver lobe, without the visualization of large vessels, liver capsule, ligaments, and the gallbladder [36]. Obesity, which is one of the most prevalent findings in NAFLD patients, might limit a successful exam in addition to poor acoustic window or presence of artifacts and inability of the subjects to hold their breath [37].

In a study that compared the diagnostic performances of supersonic shear imaging (SWE) for the diagnosis of liver fibrosis compared to ARFI and TE in chronic liver disease, SWE, TE, and ARFI correlated significantly with histological fibrosis score; AUROCs of SWE, TE, and ARFI were 0.89, 0.86, and 0.84 for the diagnosis of mild fibrosis; 0.88, 0.84, and 0.81 for the diagnosis of significant fibrosis; 0.93, 0.87, and 0.89 for the diagnosis of severe fibrosis; and 0.93, 0.90, and 0.90 for the diagnosis of cirrhosis, respectively. Hence, all methods might be used to assess liver fibrosis in patients with NAFLD, since the reliability criteria are respected as well as its limitations [37]. Additional studies with 2D-SWE and ARFI are needed in order to better establish the best cutoffs for these methods.

NAFLD and T2DM Interplay

In order to better understand the interplay between NAFLD and T2DM, it is important to review epidemiological data and pathogenetic mechanisms accounting for this relationship. As discussed before, T2DM is a risk factor for NAFLD and its progressive form, NASH, and advanced liver fibrosis [38–40]. Interestingly, in addition to T2DM, a family history of diabetes was independently associated with the presence of NASH and fibrosis in NAFLD patients [41].

In our cross-sectional study, no diabetes-related variable (glycemic control, diabetes duration, or the presence of long-term complications) was associated with the more severe stages of NAFLD [40]. In contrast, data are emerging to suggest that the presence and severity of NAFLD may be associated with the occurrence of macro and microvascular complications in diabetic patients [42–45]. Two hypotheses could explain those conflicting epidemiological observations. First, NAFLD and T2DM may represent two distinct outcomes from insulin resistance, and, in this way, no diabetes-related characteristic would be expected to favor NAFLD progression. The second hypothesis is that both NAFLD and degenerative complications may precede the diagnosis of T2DM, running over time the same and progressive course.

It has demonstrated that over 85% of subjects with NAFLD have impaired glucose tolerance or T2DM by standard oral glucose tolerance test (OGTT) [46, 47]. Therefore, another issue to be considered is whether NAFLD is an important precondition for the development of T2DM. In this regard, several studies have shown an increased incidence of T2DM in patients with NAFLD diagnosed by ultrasonography or only by elevated liver enzymes. However, most of them were conducted in Asian countries, and few were properly adjusted for potential confounding variables [48, 49]. In a recent prospective cohort study of 3153 participants from the Multi-Ethnic Study of Atherosclerosis (MESA), high liver fat was independently associated with development of T2DM [50]. Two systematic reviews with different criteria for selecting studies of NAFLD patients obtained similar results. The two independent reviews demonstrated an increased risk for incident diabetes over a period of 4–10 years [51, 52]. Taken together, these observations have implied a role for NAFLD in T2DM pathogenesis.

Pathogenesis

During the course of human evolution, individuals who had more energy stores were more likely to cope with starvation. In modern industrialized societies, with unlimited access to caloric food, this evolutionary adaptation becomes maladaptive. An increased caloric intake exceeding rates of caloric expenditure promotes obesity, dysfunction of white adipose tissue, and accumulation of ectopic lipids. This relationship between the nutritional oversupply and NAFLD is reflected by the high prevalence of NAFLD and insulin resistance (IR) among obese individuals.

Insulin Resistance

There is strong evidence of an association of NAFLD and insulin resistance (IR). Euglycemic hyperinsulinemic clamp studies, coupled with tracer infusion, confirmed that the IR is the rule in main tissues even in non-diabetic and non-obese patients with NAFLD [53]. Insulin is a pleiotropic hormone that regulates different cell functions. Concerning lipid-related metabolism, insulin promotes triglyceride storage and inhibits lipoprotein lipase activity in adipose tissue. Insulin resistance at the level of the adipocyte seems to be the primary defect in NAFLD [54, 55]. Impairment in insulin-mediated suppression of lipolysis leads first to elevated circulating non-esterified fatty acids (NEFAs) and subsequently to a sustained excess delivery of these fatty acids to skeletal muscle and liver. In fact it is the tissue-specific distribution of fat from adipose tissue into ectopic depots that determines liver and muscle insulin resistance, not the whole-body quantity of fat.

As obesity and the deposition of ectopic fat increases, adipose tissue is more likely to be infiltrated with macrophages and undergo inflammation. Thus, the expanded and dysfunctional adipose tissue secretes inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin 6 (IL-6), which decrease insulin sensitivity at the level of the adipocyte. TNFα activates pro-inflammatory pathways: the nuclear factor κB (NF-κB) and c-Jun N-terminal kinase (JNK) [56]. TNFα-induced attenuation of insulin signaling is mediated by JNK and occurs via serine phosphorylation of insulin receptor substrate [57]. In addition, serum levels of adiponectin, a hepatoprotective adipokine, are reduced in patients with NAFLD. Adiponectin improves insulin sensitivity and decreases both steatosis and inflammation [58].

Insulin resistance also leads to adverse effects on the metabolism of carbohydrates: increased gluconeogenesis and glycogenolysis in liver as well as reduction in peripheral glucose uptake. Chronic hyperglycemia induces insulin secretion by the pancreatic beta islet cells, leading to compensatory hyperinsulinemia. The mechanisms of beta cell progressive failure are less well defined; however, elevated levels of glucose as well as increased circulating NEFAs may be responsible for pancreatic beta islet cell dysfunction and apoptosis [59]. With time, as hyperglycemia worsens, fasting and total insulin production begins to decline. It is the progressive loss of beta cell insulin secretion in the setting of insulin resistance (IR)/hyperinsulinemia that predisposes to T2DM development.

Compensatory Hyperinsulinemia

Interestingly, it has been argued that many of the adverse effects due to insulin resistance result much more from compensatory hyperinsulinemia in organs that remain sensitive to its action. In fact, there is selective insulin resistance even in different pathways within the same tissue or organ. In the liver, for instance, while insulin fails to suppress gluconeogenesis, it continues to promote FAs synthesis (de novo lipogenesis, DNL). Both hyperglycemia and hyperinsulinemia activate transcription factors sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP), which upregulates most genes involved in DNL [60].

The synthesis of long-chain FAs is determined by the sequential action of various enzymes: acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), fatty acid elongases, and desaturases [61]. In turn, many of these enzymes are directly controlled by the key regulator SREBP-1c and liver

x receptor, which is also an important component of the nuclear receptor superfamily.

Hence, the action of these upregulated lipogenic enzymes and the increased delivery and uptake of FAs (adipose tissue and diet) play a critical role in the induction of NAFLD. Hepatic steatosis develops when the balance between hepatic triglycerides (TAGs) synthesis from free fatty acids (FAs) exceeds the liver capacity to oxidize FAs or export TG in the form of very-low-density lipoprotein (VLDL). FAs may be oxidized in the mitochondria, peroxisomes, and microsomal system. β-oxidation within mitochondria, however, is the most efficient source of energy under normal circumstances. Uptake and oxidation of FAs by mitochondria are both inhibited by a key intermediary of de novo lipogenesis. Decreased disposal of FFA is also the result of reduction in production of apolipoprotein B, leading to a relative impairment of very low-density lipoprotein (VLDL) generation [62].

Hepatic Insulin Resistance

Although hepatic TAGs are thought to be inert or even protective for NAFLD progression, FAs metabolites such as diacylglycerol (DAG) may further contribute to IR and NASH development. The probable causal link between citossolic DAG content and IR is attributed to PKCε activation. Activated PKCε isoform binds and inhibits insulin receptor kinase, leading to reductions in insulin-stimulated tyrosine phosphorylation of insulin receptor substrate IRS-2 and insulin signaling [63]. In contrast to hepatic DAG, other FAs metabolites, such as ceramides, are less important lipid mediators of hepatic IR in NAFLD [64].

Selective insulin resistance (IR) in the liver is a key pathophysiologic event in the development of NAFLD and type 2 diabetes. Differences in insulin receptor (InsR) activation underlie the selective IR of glucose production relative to lipogenesis. Decreased (InsR) activation has been observed in the liver of patients with NAFLD and results from a cell-autonomous downregulation of receptor number and/or activity in response to chronic hyperinsulinemia. It has been shown that a greater degree of intact InsR signaling is required to suppress glucose production than to stimulate lipogenesis, one through forkhead O transcription factor-1 (FOXO1) and the other through SREBP-1c [65]. This "bifurcation" of hepatocyte insulin signaling underlies the mechanisms by which one branch (i.e., glucose metabolism) becomes resistant to the effects of insulin, whereas the other (i.e., lipid metabolism) remains sensitive or even stimulated by hyperinsulinemia. These molecular features of hepatocyte insulin signaling do not rule out the role of excess citossolic DAG in hepatic IR.

Genetics in NAFLD Pathogenesis

During the last years, genome-wide association studies revealed genetic variants associated with NAFLD pathogenesis. Patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily 2 (TM6SF2), and glucokinase regulator (GCKR) gene polymorphisms were recently validated in large and independent cohorts. The most well-described genetic risk variant is the I148M variant in PNPLA3 gene. The I148M allele leads to a loss of phospholipase lipolytic activity, which predisposes to increased hepatic fat content and progressive liver damage. In the case of TM6SF2, (Lys E167K) T allele has been associated with hepatic retention of TAG and hepatic fibrosis and (Glu E167K) C allele with VLDL secretion and atherogenesis. However, both genetic variants in PNPLA3 and TM6SF2 have not been associated with IR or increased risk of T2DM [66, 67]. The P446L variant in GCKR gene increases glucose uptake and DNL in hepatocytes. In this setting, hepatic lipid accumulation from constant glucose substrate favors liver disease but protects from T2DM development.

Endoplasmic Reticulum Stress and the Innate Immune Response

In normal conditions, the majority of secreted and membrane proteins are folded in the endoplasmic reticulum (ER) and transported to the Golgi apparatus. In circumstances of elevated circulating (NEFAs), unfolded proteins can accumulate in this organelle. The "unfolded protein response" [68] or "the ER stress response" (ER) arises from an increased amount of unfolded proteins and impaired capacity to properly fold these proteins in the ER. Accumulation of unfolded proteins in the ER activates transmembrane signal transducers, regulates lipogenesis, and, ultimately, leads to apoptosis-inducing pathways and cell death. Furthermore, a stress-specific transcription factor named X-Box binding protein 1 (XBP1) can also activate c-Jun N-terminal (JNKs) signaling, contributing to the development of IR.

Toll-like receptors (TLRs) are a family of receptors that plays a critical role in innate immune systems. TLR4 received a particular attention because of its ability to recognize free fatty acids and lipopolysaccharides (LPSs) and activate the pro-inflammatory signaling pathway nuclear factor κB (NFκB). Thus, LPSs have an indirect effect on insulin sensitivity and inflammation [69, 70].

Patients with NAFLD have small intestinal bacterial overgrowth and increased intestinal permeability, allowing LPSs and other products to enter the portal circulation. These observations have implied an important role for gut microbiota-induced inflammation in the development of NAFLD and insulin resistance [71, 72].

Conclusion

NAFLD is present in over 30% of the world population. NASH with fibrosis may progress to cirrhosis and hepatocellular carcinoma. In addition, the recent alarming rise of obesity, T2DM and components of metabolic syndrome are even more concerning. Regarding NAFLD and T2DM interplay, it is important to highlight that T2DM is a risk factor for NAFLD and its progressive form: NASH with fibrosis. This link would explain why NAFLD in diabetic patients presents with a high prevalence of advanced stages of the disease. As discussed before, there are a wide range of non-invasive methods available for the detection of steatosis and fibrosis. In general, NAFLD score and transient elastography are the most routinely used tools to discriminate patients at risk for advanced fibrosis. However, the major challenge is to identify reliable non-invasive methods in the specific population of T2DM patients.

Another issue addressed in this chapter is the independent contribution of NAFLD to new-onset T2DM. In fact, NAFLD is believed to concur to the pathogenesis of T2DM through multiple mechanisms. The practical implication of this close interaction of NAFLD and T2DM is related to its therapeutic potential. New promising drugs in the pipeline are expected to improve NASH with fibrosis. Besides that, these new therapies may end up decreasing the risk of incident T2DM.

Multiple Choice Questions

1. Different phenotypes of non-alcoholic fatty liver disease include:
   (a) Simple steatosis, steatohepatitis, and fibrosis
   (b) Asthma COPD overlap syndrome, frequent exacerbators, and alpha-1 antitrypsin deficiency
   (c) Dominant allele and recessive allele
2. Non-alcoholic fatty liver disease is recently one of the leading causes of:
   (a) Chronic liver disease in the occident due to obesity-related epidemic and metabolic syndrome
   (b) Infections H1n1 flu virus
   (c) Gestational diabetes mellitus
   (d) Autoimmune destruction of pancreatic β cells
3. Selective insulin resistance in the liver is a key pathophysiologic event in the development of:
   (a) Non-alcoholic fatty liver disease and type 2 diabetes mellitus
   (b) Latent autoimmune diabetes in adults
   (c) Chronic obstructive pulmonary disease
   (d) Cardiovascular disease
4. Which is the first step to confirm the diagnosis when non-alcoholic fatty liver disease is suspected?
   (a) Exclude other known etiologies of chronic liver diseases like drug-related steatosis, viruses, and alcohol.
   (b) Receive an injection of a small amount of radioactive material; it flows through bloodstream and collects in certain bones or organs. A machine called a scanner detects and measures the radioactivity.
   (c) An oral glucose tolerance test measures blood sugar after you have gone at least 8 hours without eating and 2 hours after you drink a glucose-containing beverage.
   (d) Measured with a device known as a sphygmomanometer, which consists of a stethoscope, arm cuff, dial, pump, and valve.
5. Non-alcoholic fatty liver disease is defined as:
   (a) The presence of macrovesicular steatosis in ≥5% of hepatocytes in individuals who consume little or no alcohol
   (b) The presence of macrovesicular steatosis in −5% of hepatocytes in individuals who consume little or no alcohol
   (c) The presence of macrovesicular steatosis in ≥50% of hepatocytes in individuals who consume little or no alcohol
   (d) The presence of macrovesicular steatosis in ≤5% of hepatocytes in individuals who consume little or no alcohol
6. Non-alcoholic fatty liver disease is multifactorial, related to sedentarism, Western lifestyle worldwide, obesity, as well as to genetic factors with a frequency of:
   (a) Almost −20% of the general population
   (b) Almost 100% of the general population
   (c) Almost 30% of the general population
   (d) Almost 2% of the general population
7. Insulin resistance at the adipocyte level is the primary defect in non-alcoholic fatty liver disease because:
   (a) Insulin promotes HDL cholesterol storage and promotes lipoprotein lipase activity in adipose tissue.
   (b) Insulin promotes triglyceride storage and inhibits lipoprotein lipase activity in adipose tissue.
   (c) Insulin resistance is a promoter of weight loss.
8. Hyperglycemia and hyperinsulinemia activate transcription factors sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP), which upregulates most genes involved in DNL.
   (a) True
   (b) False
9. Patients with non-alcoholic fatty liver disease have small intestinal bacterial overgrowth and increased intestinal permeability, allowing LPSs and other products to enter the portal circulation.
   (a) True
   (b) False

10. Type 2 diabetes mellitus is a risk factor for non-alcoholic fatty liver disease and its progressive form, NASH, with fibrosis because:
    (a) There is an established link between diabetes and non-alcoholic fatty liver disease.
    (b) Positive family history of gestational diabetes mellitus and higher parity are established risk factors for the development of gestational diabetes mellitus.
    (c) Subjects with normal glucose metabolism (at various ages and at risk for all forms of diabetes) have shown that normal glucose tolerance is characterized by glucose levels within a very narrow range.
    (d) Physical inactivity is also a leading risk factor for the development of non-communicable diseases and is responsible for substantial economic burdens worldwide.

Correct Answers

1. (a) Simple steatosis, steatohepatitis, and fibrosis
2. (a) Chronic liver disease in the occident due to obesity-related epidemic and metabolic syndrome
3. (a) Non-alcoholic fatty liver disease and type 2 diabetes mellitus
4. (a) Exclude other known etiologies of chronic liver diseases like drug-related steatosis, viruses, and alcohol
5. (a) The presence of macrovesicular steatosis in ≥5% of hepatocytes in individuals who consume little or no alcohol
6. (c) Almost 30% of the general population
7. (b) Insulin promotes triglyceride storage and inhibits lipoprotein lipase activity in adipose tissue
8. (a) True
9. (a) True
10. (a) There is an established link between diabetes and non-alcoholic fatty liver disease

References

1. Piscaglia F, Svegliati-Baroni G, Barchetti A, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. Hepatology (Baltimore, Md). 2016;63:827–38.
2. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA. 2015;313:2263–73.
3. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology. 2015;148:547–55.
4. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980;55:434–8.
5. Kleiner D, Brunt E. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. Semin Liver Dis. 2012;32:3–13.
6. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology (Baltimore, Md). 2004;40:1387–95.
7. Loomba R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol. 2013;10:686.
8. Hoofnagle JH, Van Natta ML, Kleiner DE, et al. Vitamin E and changes in serum alanine aminotransferase levels in patients with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2013;38:134–43.
9. Babusik P, Bilal M, Duris I. Nonalcoholic fatty liver disease of two ethnic groups in Kuwait: comparison of prevalence and risk factors. Med Princ Pract. 2012;21:56–62.
10. Mendez-Sanchez N. Non alcoholic fatty liver disease. Ann Hepatol. 2009;8(Suppl 1):S3.
11. Onyekwere CA, Ogbera AO, Balogun BO. Non-alcoholic fatty liver disease and the metabolic syndrome in an urban hospital serving an African community. Ann Hepatol. 2011;10:119–24.
12. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010;28:155–61.
13. Leite N, Salles G, Araujo A, Villela-Nogueira C, Cardoso C. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver Int. 2009;29:113–9.
14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003;26(Suppl 1):S5–20.
15. Williamson RM, Price JF, Glancy S, et al. Prevalence of and risk factors for hepatic steatosis and nonalcoholic fatty liver disease in people with type 2 diabetes: the Edinburgh type 2 diabetes study. Diabetes Care. 2011;34:1139–44.
16. Sanyal A, Poklepovic A, Moyneur E, Barghout V. Population-based risk factors and resource utilization for HCC: US perspective. Curr Med Res Opin. 2010;26:2183–91.
17. Charlton M, Burns J, Pedersen R, Watt K, Heimbach J, Dierkhising R. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology. 2011;141:1249–53.
18. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123:745–50.
19. Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366:1059–62.
20. Jeanes YM, Reeves S. Metabolic consequences of obesity and insulin resistance in polycystic ovary syndrome: diagnostic and methodological challenges. Nutr Res Rev. 2017;30(1):97–105.
21. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002;346:1221–31.
22. Osman KA, Osman MM, Ahmed MH. Tamoxifen-induced non-alcoholic steatohepatitis: where are we now and where are we going? Expert Opin Drug Saf. 2007;6:1–4.
23. Farrell GC. Drugs and steatohepatitis. Semin Liver Dis. 2002;22:185–94.
24. Debongnie JC, Pauls C, Fievez M, Wibin E. Prospective evaluation of the diagnostic accuracy of liver ultrasonography. Gut. 1981;22:130–5.
25. Scaglioni F, Ciccia S, Marino M, Bedogni G, Bellentani S. ASH and NASH. Dig Dis. 2011;29:202–10.
26. Liangpunsakul S, Chalasani N. What should we recommend to our patients with NAFLD regarding alcohol use? Am J Gastroenterol. 2012;107:976–8.

27. Pendino GM, Mariano A, Surace P, et al. Prevalence and etiology of altered liver tests: a population-based survey in a Mediterranean town. Hepatology (Baltimore, Md). 2005;41:1151–9.
28. Abdelmalek MF, Sanderson SO, Angulo P, et al. Betaine for non-alcoholic fatty liver disease: results of a randomized placebo-controlled trial. Hepatology. 2009;50:1818–26.
29. Sorrentino P, Tarantino G, Conca P, et al. Silent non-alcoholic fatty liver disease-a clinical-histological study. J Hepatol. 2004;41:751–7.
30. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352:854–65.
31. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59:1265–9.
32. Bedossa P. Pathology of non-alcoholic fatty liver disease. Liver Int. 2017;37(Suppl 1):85–9.
33. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285:2486–97.
34. de Ledinghen V, Wong VW, Vergniol J, et al. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan(R). J Hepatol. 2012;56:833–9.
35. Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology (Baltimore, Md). 2010;51:454–62.
36. Samir AE, Dhyani M, Vij A, et al. Shear-wave elastography for the estimation of liver fibrosis in chronic liver disease: determining accuracy and ideal site for measurement. Radiology. 2015;274:888–96.
37. Cassinotto C, Lapuyade B, Mouries A, et al. Non-invasive assessment of liver fibrosis with impulse elastography: comparison of Supersonic Shear Imaging with ARFI and FibroScan(R). J Hepatol. 2014;61:550–7.
38. Younossi Z, Gramlich T, Matteoni C, Boparai N, Mccullough A. Nonalcoholic fatty liver disease in patients with type 2 diabetes. Clin Gastroenterol Hepatol. 2004;2:262–5.
39. Prashanth M, Ganesh HK, Vima MV, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. J Assoc Physicians India. 2009;57:205–10.
40. Leite N, Villela-Nogueira C, Pannain V, et al. Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. Liver Int. 2011;31:700–6.
41. Loomba R, Abraham M, Unalp A, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology (Baltimore, Md). 2012;56:943–51.
42. Targher G, Bertolini L, Padovani R, et al. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30:1212–8.
43. Targher G, Bertolini L, Padovani R, et al. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. J Hepatol. 2010;53:713–8.
44. Targher G, Bertolini L, Rodella S, Lippi G, Zoppini G, Chonchol M. Relationship between kidney function and liver histology in subjects with nonalcoholic steatohepatitis. Clin J Am Soc Nephrol. 2010;5:2166–71.
45. Targher G, Chonchol M, Pichiri I, Zoppini G. Risk of cardiovascular disease and chronic kidney disease in diabetic patients with non-alcoholic fatty liver disease: just a coincidence? J Endocrinol Investig. 2011;34:544–51.
46. Ortiz-Lopez C, Lomonaco R, Orsak B, et al. Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). Diabetes Care. 2012;35:873–8.
47. Willner I, Waters B, Patil S, Reuben A, Morelli J, Riely C. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. Am J Gastroenterol. 2001;96:2957–61.
48. Kasturiratne A, Weerasinghe S, Dassanayake AS, et al. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. J Gastroenterol Hepatol. 2013;28:142–7.
49. Choi JH, Rhee EJ, Bae JC, et al. Increased risk of type 2 diabetes in subjects with both elevated liver enzymes and ultrasonographically diagnosed nonalcoholic fatty liver disease: a 4-year longitudinal study. Arch Med Res. 2013;44:115–20.
50. Shah RV, Allison MA, Lima JA, et al. Liver fat, statin use, and incident diabetes: the multi-ethnic study of atherosclerosis. Atherosclerosis. 2015;242:211–7.
51. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43:617–49.
52. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016;31:936–44.
53. Bugianesi E, Gastaldelli A, Vanni E, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia. 2005;48:634–42.
54. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology. 2012;142:711–25.e6.
55. Lomonaco R, Ortiz-Lopez C, Orsak B, et al. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology. 2012;55:1389–97.
56. Fain JN, Bahouth SW, Madan AK. TNF alpha release by the non-fat cells of human adipose tissue. Int J Obes Relat Metab Disord. 2004;28:616–22.
57. Sabio G, Das M, Mora A, et al. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. Science. 2008;322:1539–43.
58. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism. 2011;60:313–26.
59. Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes. 2005;54(Suppl 2):S97–107.
60. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002;109:1125–31.
61. Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab. 2008;34:643–8.
62. Ferre P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. Diabetes Obes Metab. 2010;12(Suppl 2):83–92.
63. Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 2014;59:713–23.
64. Kumashiro N, Erion DM, Zhang D, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. Proc Natl Acad Sci U S A. 2011;108:16381–5.

65. Cook JR, Langlet F, Kido Y, Accili D. Pathogenesis of selective insulin resistance in isolated hepatocytes. J Biol Chem. 2015;290:13972–80.
66. Pirola CJ, Sookoian S. The dual and opposite role of the TM6SF2-rs58542926 variant in protecting against cardiovascular disease and conferring risk for nonalcoholic fatty liver: a meta-analysis. Hepatology. 2015;62:1742–56.
67. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology. 2011;53:1883–94.
68. Czupryniak L. Guidelines for the management of type 2 diabetes: is ADA and EASD consensus more clinically relevant than the IDF recommendations? Diabetes Res Clin Pract. 2009;86(Suppl 1):S22–5.
69. Konner AC, Bruning JC. Toll-like receptors: linking inflammation to metabolism. Trends Endocrinol Metab. 2011;22:16–23.
70. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56:1761–72.
71. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology. 2009;49:1877–87.
72. Ruiz AG, Casafont F, Crespo J, et al. Lipopolysaccharide-binding protein plasma levels and liver TNF-alpha gene expression in obese patients: evidence for the potential role of endotoxin in the pathogenesis of non-alcoholic steatohepatitis. Obes Surg. 2007;17:1374–80.