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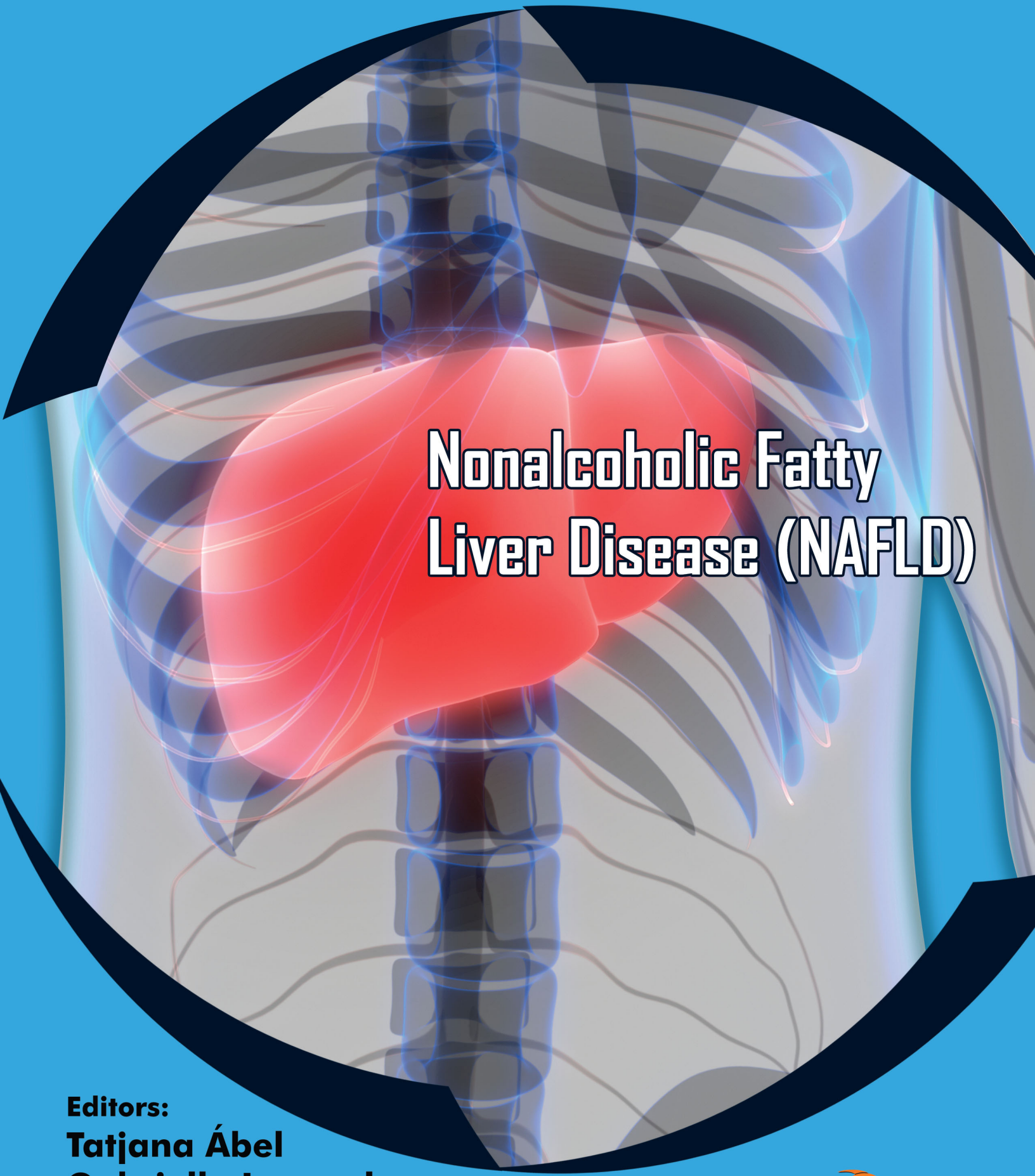

Nonalcoholic Fatty Liver Disease (NAFLD)

Editors:
Tatjana Ábel
Gabriella Lengyel

Bentham Books

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FOREWORD

This book has attempted to deal with the non-alcoholic fatty liver disease (NAFLD) as well as the pathogenesis of it. Hepatocellular carcinoma (HCC) is a well-known complication of NASH, therefore, the prevention and treatment of NAFLD and its complications as early as possible have an outstanding importance. NAFLD refers to a wide spectrum of liver injury, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis. NAFLD is associated with insulin resistance, type 2 diabetes mellitus.

This e-book has 9 chapters, where the diagnosis of NAFLD was detected either by imaging or histologically. They can be used to follow up the progression of the disease, monitor the efficacy of potential therapies and compare different studies.

One of the chapters deals with ultrasound (the non-invasive method), having a sensitivity of approximately 85% and specificity of 94% for the detection of moderated fatty liver. Magnetic resonance imaging (MRI) and surrogate markers Fatty Liver Index (FLI) have gained attention.

After reviewing these topics, the chapter provides a brief overview of the clinical characteristics, screening, and novel opportunities in the chemoprevention of NAFLD-related HCC. Special MRI sequences (Chemical Shift Imaging, Fast SE Imaging, elastography, spectroscopy) are capable of providing comparable results on biopsy. In contrast to biopsies, these methods provide a non-invasive way of giving a representative assessment of the whole liver. Additional therapeutic possibilities of the future may target antioxidant defense, immune–mediated mechanisms, apoptosis, and lipogenesis.

NAFLD has high prevalence in obese children, which has serious consequences without treatment.

This e-book draws the attention towards the fact that early intervention is most important when NAFLD is diagnosed, which should include early lifestyle modification (nutrition and physical activity, avoidance of smoking).

I feel that the students and medical doctors should have some Knowledge of these important concepts. I believe this is necessary in view of the importance of this system in clinical medicine.

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PREFACE

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of elevated liver enzymes and chronic liver disease in Western countries. Patients with elevated liver enzymes in the absence of alcohol consumption and secondary causes of liver disease are described as having NAFLD, which is an independent predictor of future risk of cardiovascular diseases, type 2 diabetes and metabolic syndrome (hypertension, abdominal obesity, dyslipidemia, glucose intolerance). The 'two hit' theory introduced in 1998 by Day and James proposes that development of NASH requires a second hit to an already sensitized liver by steatosis which constitutes the first hit. Recently, the 'two hit' theory was strongly challenged and 'one-hit' and 'multiple-hit' theories have been proposed. Insulin resistance and obesity are two important factors of pathogenesis of NAFLD. The pathomechanism of NAFLD involves multiple genetic and environmental factors. Besides the genetic susceptibility to develop the disease, it appears that promoting factors notably include: lipid intermediate accumulation, altered expression of pro-inflammatory cytokines and mitochondrial dysfunction.

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CHAPTER 1

Epidemiology of NAFLD

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Abstract: Non-alcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver injuries, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis. NAFLD is associated with insulin resistance, type 2 diabetes mellitus, obesity, hypertriglyceridemia and hypertension; thus, it is regarded as a hepatic component of the metabolic syndrome, and an independent risk factor for cardiovascular disease.

NAFLD and NASH are common causes of chronic liver disease and elevated liver enzymes. Their worldwide prevalence continues to increase with the growing obesity epidemic. Understanding the epidemiology of these pathologies is essential for developing treatment and prevention strategies. The prevalence of NAFLD and NASH in the general population has been assessed with a variety of diagnostic means, such as liver biopsy, non-invasive radiological and ultrasonic techniques, elevated liver enzymes and combinations of clinical variables. Because liver biopsy is not appropriate for population studies, only on the basis of autopsy studies it has been suggested that 3-5% of individuals in the general population might have NASH, and 20-30% of people in industrialized countries have NAFLD. The prevalence of NAFLD increases with age, it is highest in males between 40-65 years and is higher in Hispanics and lower in African-Americans.

Ultrasound is the non-invasive method most commonly used to assess NAFLD, having a sensitivity of approximately 85% and a specificity of 94% for the detection of moderated fatty liver. *Magnetic resonance imaging (MRI)* has also been used to perform population studies, but it is less portable and more expensive than US. Among *surrogate markers Fatty Liver Index* (FLI) has gained much attention.

Several studies have shown that NASH is a risk factor for liver fibrosis. At the same time, most cases of fatty liver and even fibrosis can regress, particularly due to life style modification and weight loss. Based on the well-established strong association of the NAFLD with the metabolic syndrome and the epidemic of obesity, the prevalence of NASH is expected to increase in the next decade, leading to cirrhosis and even HCC. There is a need to perform larger, longitudinal studies that assess the long-term natural

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history of NAFLD with validated non-invasive biomarkers and by integrating morbidity and mortality data.

Keywords: Epidemiology, Natural history, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis, Prevalence, Risk factors.

INTRODUCTION

Over the past 5 years several comprehensive reviews studied the epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). Among them, *Byrne et al.* [1] when discussing the metabolic disturbances in NAFLD, emphasized that since obesity and diabetes are increasing in prevalence worldwide, there is a marked increase in NAFLD, which occurs in individuals of all ages and ethnic groups, and at the same time, it is an independent risk factor for cardiovascular disease (CVD). *Cheung* and *Sanyal* also underlined that NAFLD is the most common cause of chronic liver disease in North America and some Western countries associated with the metabolic syndrome, and its prevalence is estimated to be as high as 35% in some populations linked to the growing epidemic of obesity [2]. *Ratziu et al.,* based on the EASL 2009 special conference, outlined the concepts on NAFLD, as an increasingly relevant public health issue. Since recently NAFLD is a frequent condition, it often co-exists with other chronic liver diseases, such as alcohol abuse, and HCV infection or hemochromatosis, EASL suggested a shift in diagnostic concept of NAFLD from a "negative" diagnosis of exclusion (to drop the "negative" definition of "nonalcoholic") to a positive one, based on the presence of an underlying condition, instead of the absence of an unrelated disease. Thus, according to EASL, there would be a need for a change in nomenclature, discarding the negative definition, and using a name of "*metabolic steatosis or metabolic steatohepatitis*" [3]. In their overview, *Lewis* and *Mohanty* pointed out that NAFLD affects approximately 20-30% of population in industrialized countries, males and females are both equally affected; however, some studies revealed that it is more common in men than women in morbidly obese and Asian populations [4]. In 2011, *Vernon et al.* in a paper summarized the epidemiology and natural history of NAFLD based on clinical literature published over the past 30 years. They emphasized that understanding the epidemiology of NAFLD is essential for developing effective treatment and prevention strategies, which is of pivotal importance, because the prevalence of NAFLD and NASH is expected to increase in the next decade, leading to advanced fibrosis, cirrhosis and even HCC [5]. *Bhatia et al.* [6] provided a state-of-art article of the evidence linking NAFLD with CVD, the potential mechanisms underlying this association,

its relation to insulin resistance and the metabolic syndrome, in the context of increased CV risk. The role of NASH as a potential independent CV risk factor has gained considerable importance such as an awareness of this disease is essential for practicing cardiologists, given that it affects 20-30% of general population. *Weiß et al.* [7] when reviewing the publications on NAFLD that appeared between 1995 and 2013 stated that at present some 5% to 20% of patients with NAFLD develop NASH, and fewer than 5% of cases progress to cirrhosis. Approximately 0.05% to 0.3% may be the prevalence of cirrhosis in the general population and 2% of cirrhotic patients per year develop HCC. Recently *Bedogni et al.* provided a concise overview of the epidemiology of NAFLD published between January 2011 and October 2013; they noted, that NAFLD may be a separate entity, rather than an additional component of metabolic syndrome, but it remains to be tested, whether metabolic syndrome and NAFLD contribute to "hard outcomes" in the general population independently [8].

PREVALENCE OF NAFLD AND NASH

The prevalence of NAFLD and NASH in general population depends on the diagnostic methods, such as liver biopsy, radiological and ultrasonic techniques, elevated liver enzymes, and combination of clinical variables.

Liver Biopsy and Autopsy Studies

Liver biopsy (LB) has long been regarded as gold standard for the diagnosis and staging of NAFLD and NASH, though it is an invasive measure and cannot be used in population-based studies. Yet, valuable findings have been reported for liver **transplant donors** who are considered healthy people. A Korean study in which LB-s were performed on 589 consecutive potential liver transplant donors reported NAFLD prevalence of 51% [9].

In the USA 20% of donors were ineligible for donation based on the degree of steatosis (>30%) [10]. A high prevalence of histological NAFLD has been described in healthy living liver donors: 12-18% in Europe [11, 12], and 27-38% in the USA [13, 14]. In healthy living liver donors, the prevalence of NASH ranges from 3% to 16% in Europe [11, 12], and from 6% to 15% in the USA [13, 14].

The results of **autopsy** series showed mixed data regarding the prevalence of NAFLD. In an autopsy series of lean individuals from Canada, the prevalence of NASH and fibrosis was 3% and 7%, respectively [15], while a study from Greece showed evidence of steatosis in 31% and NASH in 40% of autopsied cases of

CHAPTER 2

Clinical Manifestations and Diagnosis of NAFLD

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Abstract: The non-alcoholic fatty liver disease (NAFLD) is a significantly increasing cause of chronic liver disease which is strongly associated with obesity and insulin resistance. Due to this association it is considered as the hepatic manifestation of the metabolic syndrome. According to the emerging clinical and epidemiological data patients with NAFLD have an increased morbidity and mortality of cardiovascular diseases (CVD), type 2 diabetes mellitus, chronic kidney disease as well as malignancies, beyond the liver-related mortality. A number of other less established comorbidities can also manifest together with NAFLD, like colorectal cancer, hypothyroidism, obstructive sleep apnea, polycystic ovarian syndrome and osteoporosis.

The majority of the patients however, maybe asymptomatic and the diagnosis is made only incidentally. Obesity, high body mass index (BMI), elevated transaminase levels and/or hyperechogenic ultrasound form the basis for the diagnosis. About 20% of the cases can progress to non-alcoholic steatohepatitis (NASH) and cirrhosis. Fibrosis, however, can be initiated either in simple steatosis or in NASH *i.e.* due to the most recent results, fibrosis progression is independent of the presence of NASH. In patients with simple steatosis and no inflammation, the fibrosis progression is very slow. The rapid progressors, however, can progress to cirrhosis within 2-6 years. In these patients, hypertension and diabetes are usually also present. The presence and severity of fibrosis on liver biopsy are the best indicators of long-term liver-related outcome in patients with NAFLD. The most important step during diagnosis is risk stratification.

Once a patient with NAFLD develops cirrhosis, he has the same natural history as with other etiologies. Patients with compensated cirrhosis have a 3-4% risk of mortality annually.

Keywords: Cardiovascular diseases, Chronic kidney disease, Colorectal cancer, Hypothyroidism, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis, Obstructive sleep apnea, Osteoporosis.

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INTRODUCTION

The definition of non-alcoholic fatty liver disease (NAFLD) requires two conditions: the first and most important is to detect steatosis in the liver and the other is the exclusion of other conditions leading to secondary fat accumulation in the liver, such as alcohol consumption, hepatitis C virus infection (especially genotype 3), use of teratogenic medications (tamoxifen, amiodarone *etc.*), parenteral nutrition, hereditary disorders, severe malnutrition, *etc.* (Table **1**). NAFLD encompasses the entire spectrum of fatty liver disease ranging from fatty liver (NAFL) to steatohepatitis (NASH) and cirrhosis. NAFL is described as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes or no evidence of fibrosis. The risk of progression to cirrhosis is minimal. NASH is defined as the presence of hepatic steatosis, inflammation with hepatocyte injury with or rarely without fibrosis. This can progress to cirrhosis, and later end-stage liver disease, *i.e.* liver failure and/or liver cancer [1].

Table 1. Common causes of secondary hepatic steatosis.

Excessive consumption of alcohol
Chronic hepatitis C (especially genotype 3)
Wilson's disease
Malnutrition
Parenteral nutrition
Medications (amiodarone, methotrexate, steroids, tamoxifen, valproate, *etc.*)
Hereditary lipid abnormalities (abetalipoproteinaemia, lecithin-cholesterol-acyltransferase deficiency)
Acute fatty liver of pregnancy
HELLP syndrome
Reye's syndrome

In the US, as well as in Europe, NAFL has become the most common cause of chronic liver disease. The proportion of NAFLD among chronic liver diseases rose from 47% to 75% in the last 20 years [2]. This is definitively coupled to the increase of obesity, visceral obesity, type 2 diabetes mellitus (T2DM), insulin resistance (IR) and hypertension in the population of the Western societies, together with the ageing of the population. Beyond these parameters of the metabolic syndrome, patients with NAFLD generally display a number of other co-morbidities, as is listed in Table **2**. It is very important to systematically look for these additional conditions. It is believed, that patients with more risk factors have a risk for more advanced NAFLD. A significant percentage of the patients

with NAFLD is asymptomatic and the disease is only diagnosed incidentally, especially at the early stages. Due to the co-morbidities, the clinical symptoms are quite variable. There are several extrahepatic disorders, beyond the liver disease, which can cause symptoms, like cardiovascular diseases (CVD), T2DM, chronic kidney disorders (CKD), *etc.*, and some malignant diseases are also detected at a higher rate [3]. It has been shown, that the cardiovascular mortality as well as deaths due to malignancies are both higher in patients with NAFLD, than the liver-related mortality. In patients with NAFLD the cardiovascular mortality is two-times higher than the liver related mortality. In patients with cirrhosis, the cardiovascular disease is the second cause of death. Patients with NAFLD have 9 times more chances of death than patients in the general population. Interestingly, there is no difference between the cardiovascular mortality in patients with NAFL as compared to NASH [4, 5]. NAFLD is strongly associated with obesity and metabolic syndrome, however, a small percentage of the patients also develops NAFLD despite normal body mass index (BMI). The fact, that NASH can be present in lean patients, makes this picture even more complex [6]. The details of the clinical manifestations of NAFLD, except diabetes mellitus, will be discussed in this chapter.

Table 2. Comorbidities associated with NAFLD.

Type 2 diabetes mellitus
Metabolic syndrome
Cardiovascular diseases
Chronic kidney disease
Malignant diseases
Polycystic ovarian syndrome
Chronic obstructive apnea
Hypogonadism
Hypothyroidism
Vitamin D deficiency, osteoporosis

Extrahepatic Complications of NAFLD

Cardiovascular Disorders and NAFLD

Patients affected by NAFLD have a higher risk of developing cardiovascular (CV) events and deaths [7]. One of the most important questions is whether this is only a timely correlation based on underlying risk factors that are present in either conditions, or an independent contribution of NAFLD in the development of

CHAPTER 3

Imaging of NAFLD

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Abstract: With the epidemic of obesity and metabolic syndrome, NAFLD is affecting a large number of the general population than ever before. Its diagnosis and monitoring can be challenging as it is more common in obese patients. It is a reversible condition, and reaching an early diagnosis could help in preventing and reversing the process. If left untreated, it can advance to liver cirrhosis, and lead to hepatocellular carcinoma in some cases. Therefore, its non-invasive accurate and early diagnosis has a significant importance in both patients and clinicians. Radiology has to offer a wide repertoire of methods that can diagnose and monitor this condition. Biopsy is very accurate, and is the only widely accepted method to distinguish NAFLD from other forms of liver disease, but its inconvenience to the patient and the general risks of invasive procedures limits its clinical use. In addition, biopsy cannot be representative to structural changes in the entire organ. Medical imaging until recent advances was not able to compete with biopsy. Non-invasive diagnostic tests that are used include ultrasonography (sonoelastography), computed tomography, and magnetic resonance imaging. Special MRI sequences (Chemical Shift Imaging, Fast SE Imaging, elastography, spectroscopy), which are capable of providing comparable results to biopsy. In contrast to biopsies, these methods provide a non-invasive way of giving a representative assessment of the whole liver.

Keywords: Cirrhosis, Fat suppression, Fibroscan, Hepatocellular carcinoma, Liver biopsy, Liver CT liver imaging, Liver MRI, Liver ultrasound, MR elastography, MR spectroscopy, Sonoelastography, Steatohepatitis, Steatosis, Steatosis imaging.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is nowadays the most common cause of abnormality in liver function. Its prevalence in the average population is 9-32%. During the course of the disease there is a significant focal or diffuse accumulation of triglycerides in the hepatocytes.

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Diffuse deposition of fat can be mild, or can progress to steatohepatitis, liver cirrhosis and eventually can lead to hepatocellular carcinoma.

Nowadays, NAFLD is increasingly seen in younger ages, therefore fast, accurate, and non-invasive diagnostic methods are coming to the forefront. This is important for both early diagnosis, and also in the follow-up of treatment response as well.

Biopsy is the most accurate method and is still considered to be the gold standard in determining the extent of fatty infiltration. However, its disadvantages include complications in a small number of cases (<3%). It cannot be representative of structural changes in the liver, and it cannot be repeated indefinitely. At the same time, diagnostic imaging has become more reliable to accurately detect steatosis and determine its stage without the need for any invasive procedure. The diagnostic imaging modalities available for the diagnosis of NAFLD are ultrasound (US), sonoelastography, computed tomography (CT), magnetic resonance imaging (MRI) [1].

IMAGING MODALITIES

Ultrasound

Ultrasound is the most readily available, inexpensive imaging modality, with no ionising radiation involved. A convex transducer with a frequency range of 2-8 MHz is used to examine the liver, but the frequency used is highly dependent on the patient's physique.

The assessment of the liver's echogenicity and the degree of steatosis is very subjective, and operator dependent. Ultrasound is operator dependant, and there can be large variations between examinations performed on different machines, and even a single sonographer is not always able to reproduce previous parameters. The normal liver is homogeneous, has a characteristic echotexture, the echogenicity of which equals to, or is slightly more than that of the kidney and spleen. The liver appears to be homogeneous if examined with a good quality ultrasound, the intrahepatic vessels can be sharply delineated, and the diaphragmatic surface of the liver has a well distinguishable boundary. In fatty infiltration of the liver there is an increased reflection of ultrasound waves, this will result in a "bright liver". In severe fatty infiltration due to the pronounced attenuation of sound waves, the liver structure will become increasingly difficult to assess. The degree of fatty infiltration has three grades on US.

Initially, the fatty deposition increases the echogenicity of the liver (Grade I.), then the contour of the vessels will become blurred (Grade III.), in more severe steatosis the contour of the diaphragmatic surface will become obscured or even masked (Grade III.) (Fig. **1**).

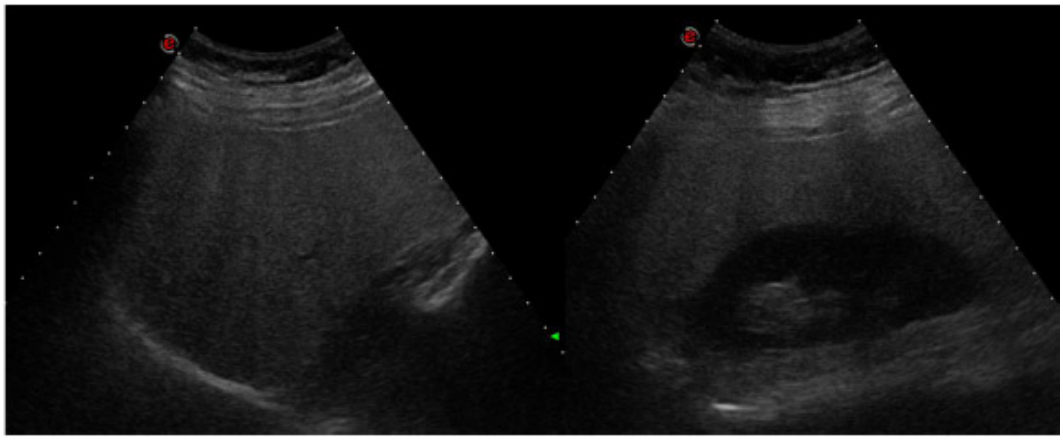

Fig. (1). On ultrasound image severe fatty infiltration of the liver cause pronounced attenuation of sound waves. The echogenicity of the liver is more higher than that of the kidney.

The sensitivity of US with respect to hepatic steatosis is between 60-94%, specificity 84-95% [2]. In the case of marked obesity both the sensitivity (49%) and specificity (75%) decrease. The sensitivity of US is almost 100% when steatosis the liver exceeds 30%, in these cases the differentiation between steatosis and fibrosis is extremely difficult [3].

Sonoelastography

The latest US techniques allow for the assessment of the livers' elasticity. This function is either built into modern US machines (sonoelastography), or a purpose built fibroscan machine can quantitatively determine the stiffness of liver parenchyma.

It is a generally known fact that diseased tissue has different elasticity compared to healthy tissue. This can partly be explained by the amount of extracellular fluid, since after dehydration almost all human tissue will become incompressible.

We can assess tissue elasticity by elastography, which has two different types; strain, and shearwave elastography. Both techniques can be used in several ways. The formers' most common method of implementation is the acoustic radiation force impulse imaging (ARFI), and the latter's is the transient elastography. In ARFI, a high energy focused ultrasound impulse wave is applied; this in turn will

CHAPTER 4

Histopathological Changes of NAFLD

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide. Although imaging techniques and serologic testing are important examinations, histology remains the gold standard to establish the diagnosis, to stratify to grade and stage the actual sample. There are two major subtypes of NAFLD, simple steatosis and the progressive form nonalcoholic steatohepatitis (NASH). Simple steatosis is characterized by fat accumulation in hepatocytes. NASH can be diagnosed if, in addition to steatosis, inflammation and hepatocyte damage in the form of ballooning is present in the liver. There may be other histological alterations with variable significance *e.g.* fibrosis, ductular reaction, granulomas, Mallory-Denk bodies, *etc*. NASH is a progressive disease, which can end up in cirrhosis. Hepatocellular carcinoma (HCC) is also a well-known complication of NASH. It can develop in cirrhotic and surprisingly in non-cirrhotic stage of NASH. The histological signs of NASH can be substantially different in pediatric patients, than in adults. Several histological scoring systems have been developed for reliable grading and staging of NAFLD. They can be used to follow up the progression of the disease, monitor the efficacy of potential therapies and to compare different studies. Future will decide which one of them proves to be most reliable, and reproducible. Finally, histological diagnosis can be important to distinguish NAFLD from other chronic liver diseases or recognize comorbidities.

Keywords: Ballooning, Cirrhosis, Ductular reaction, Hepatic fibrosis, Hepatocellular carcinoma, Mallory-Denk bodies, Scoring, Steatohepatitis, Steatosis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronically elevated levels of serum alanine transaminase (ALT) in both Western and developing societies. It is more common than viral hepatitis or alcoholic liver disease. Its exact prevalence is not known, however an estimated nearly 30% of

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the population is affected in the USA, UK or China, and it is more frequently diagnosed in children [1]. Therefore, it represents an enormous health care issue requiring further efforts to develop new strategies for its prevention and treatment.

NAFLD is defined as increased accumulation of hepatic triglyceride, steatosis, without excess alcohol consumption (<20g/day for men, <10g/day for women). It is the hepatic manifestation of metabolic syndrome and can be considered as a consequence of systemic and hepatic insulin resistance. NAFLD can manifest as a broad range of histological changes, from simple steatosis to non-alcoholic steatohepatitis (NASH). This latter form can progress into liver cirrhosis and NAFLD is more often thought to serve as a basis for the development of hepatocellular carcinoma (HCC) in cirrhotic or non-cirrhotic liver. Not only liver diseases and mortality are more common in people with NAFLD, recent epidemiologic studies indicate that cardiovascular diseases and non-hepatic forms of cancer are more common in patients with simple steatosis, while steatosis and diabetes or NASH are important risk factors for liver-related mortality [2]. Seventy per cent of centrally obese patients with hypertension and diabetes have steatohepatitis shown by liver biopsy [3]. An early diagnosis of the affected individuals is extremely important. NASH is usually considered as a condition, which is preceded by simple steatosis and insulin resistance. However, there are evidences suggesting that NASH and simple steatosis could arise as two independent alterations. This is supported by the fact that progression of steatosis into NASH is rarely observed. . Although steatosis and NASH are still considered as histological subtypes along the spectrum of NAFLD, they are likely different entities not only from histological but also from pathophysiological standpoint. The most widely used and simplest screening laboratory test for liver dysfunction is the determination of serum ALT level. This examination, however, has proven to be very unreliable in recognizing patients with NAFLD. In several well-documented studies, normal ALT levels were found even in advanced stages of the disease. In 40% of the children with varying degree of fibrosis, the ALT value was in the normal range and almost 10% of adults with NAFLD-related cirrhosis had no elevated transaminase level [4].

Imaging techniques can provide a significant help in recognizing NAFLD patients, but they are really efficient only in patients with an advanced disease when the fat content is increased in more than 33% of the hepatocytes [5]. Unfortunately, there is no available simple blood test or imaging technique that could differentiate simple steatosis from NASH. The simplest and cheapest technology, ultrasound, shows a high level of interobserver variability. The most reliable magnetic resonance imaging (MRI) is very expensive and it is not

available for screening examinations [6]. The combination of serologic and imaging markers and associated clinical algorithms has advanced to a great extent in the last few years, but still cannot replace the histological examination of liver samples, which despite its limitations remains the "gold standard" for confirming or excluding NASH in suspicious patients.

GENERAL HISTOLOGICAL FEATURES OF NAFLD

The hallmark of NAFLD is the hepatocellular steatosis or fatty change. Fatty hepatocytes may occur occasionally in normal liver, but the presence of fat in more than 5% of the hepatocytes is regarded as pathological. The NAFLD-associated fatty change is usually macrovesicular, but microvesicular form may also develop sometimes. The fatty hepatocytes appear first around the central veins in zone 3 of the hepatic acinus (Fig. **1**), but the whole lobule may be involved, depending on the severity of the process.

The severity of steatosis is usually divided into 3 categories: mild (5-33%), moderate (33-66%), and severe (>66%). The extent of steatosis shows some correlation with the activity of inflammation; patients with severe fatty changes are more likely to have NASH.

The major histopathological components of steatohepatitis are: steatosis, inflammation (lobular and portal) and the so called ballooning of hepatocytes, a morphological sign of hepatocyte damage (Table **1**).

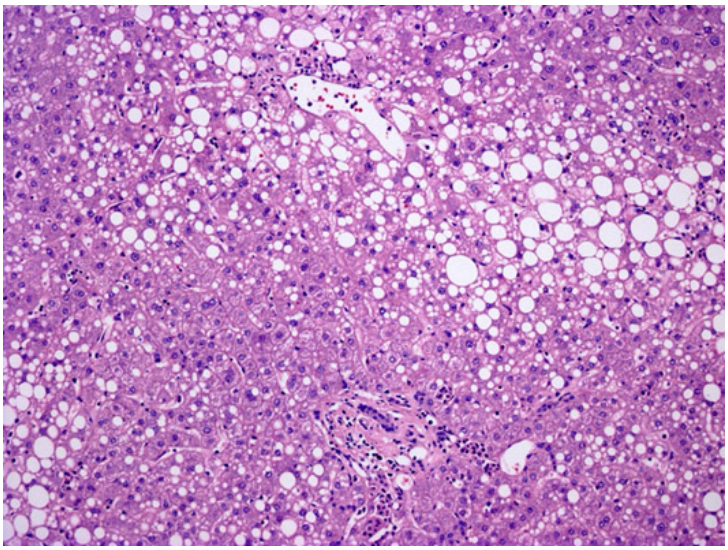

Fig. (1). The hallmark of NAFLD is fatty change. The steatotic hepatocytes have preferential pericentral (zone 3) distribution.

CHAPTER 5

Pathophysiology of NAFLD

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Abstract: *Non-alcoholic fatty liver disease* (NAFLD) and its more severe form, *non-alcoholic steatohepatitis* (NASH) are common causes of chronic liver disease and major components of the metabolic syndrome. NASH is characterized by the presence of steatosis with necro-inflammation and fibrosis, progressing to cirrhosis and hepatocellular carcinoma. The pathogenesis of NAFLD and NASH originally was regarded as "two-hit" model, suggesting that the accumulation of fat in the liver cells (steatosis) as the first sensitizes the liver to a second hit that triggers a cascade of tissue damages (necro-inflammation and fibrosis). Today, it is widely accepted, that a more complex process, involving multiple parallel metabolic hits is responsible for tissue injury, and that other factors promote disease progression. Thus, now, *lipotoxicity, mitochondrial dysfunction, insulin resistance and oxidative stress* are considered as the main mechanisms in the pathogenesis of NASH. Reactive oxygen species (ROS), lipid peroxidation products and cytokines are involved in the progression, including the migration of resident hepatic pro-fibrogenic cells, which leads to fibrosis. Hepatocyte death, inflammation, and cellular senescence also play a role in the pathogenesis of the disease. The interaction between inflammatory cells including Th17 cells and other cell types such as hepatocytes, stellate cells, hepatic progenitor cells and ductular components is of pivotal importance, as well as the reactivation of developmental morphogenic signaling pathway, the hedgehog.

Keywords: Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis, Pathogenesis.

INTRODUCTION

The pathogenesis of non-alcoholic fatty liver disease (NAFLD) and its more severe form, non-alcoholic steatohepatitis (NASH), was regarded originally as a "**two-hit**" model, suggesting that the accumulation of fat in the liver cells (steatosis) as the *first hit* sensitizes the liver to a *second hit* that triggers a cascade of tissue injuries (necro-inflammation and fibrosis) [1]. Today, it is

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widely accepted, that a more complex process, involving multiple parallel metabolic hits is responsible for tissue injury, and that other factors promote disease progression [2 - 5] (Fig. **1**).

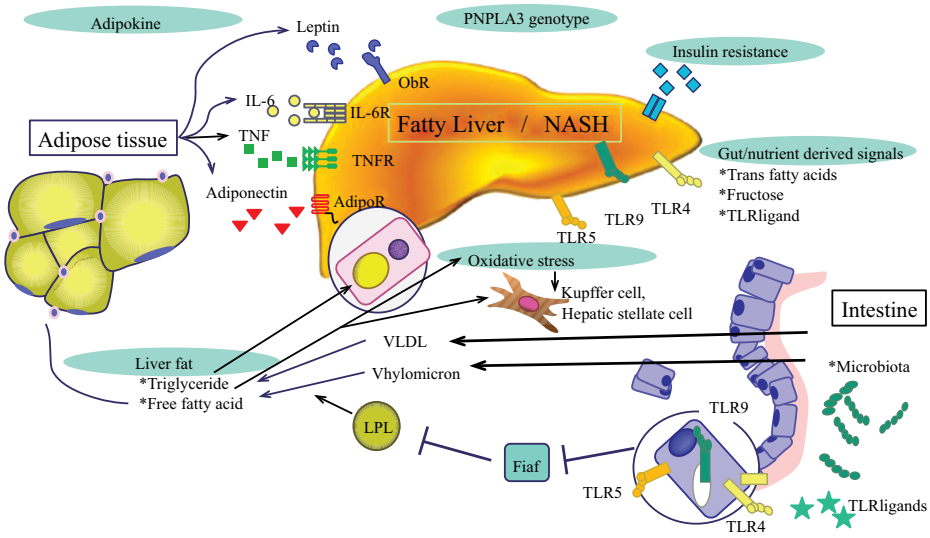

Fig. (1). Multiple parallel hit theory of NAFLD.

Genome-wide association studies have suggested the pivotal importance of patatin-like phospholipase 3 (PNPL3) gene polymorphism in NAFLD and the inflammation, that could even precede steatosis. Obesity and diabetes induce insulin resistance, adipocyte proliferation and changes in intestinal flora. Cytokines, such as IL-6 and TNF-α produced by adipocytes affect hepatocyte fat content and liver inflammatory environment. Gut derived signals are affected by ingested fatty acids, fructose, or TLR ligands. Free fatty acids and triglycerides induce ER stress and oxidative stress, resulting in inflammation and fibrogenesis in the liver.

Free fatty acids, cytokines, oxidative stress, apoptosis and gut-derived lipopolysaccharides (LPSs) trigger an *inflammatory response and liver damage* in NAFLD and NASH. *Reactive oxygen species* (ROS) generated during free fatty acid (FAA) metabolism in microsomes, peroxisomes and mitochondria are the source of oxidative stress. ROS, lipid peroxidation products and cytokines are involved in the progression of simple steatosis to NASH, *insulin resistance and mitochondrial dysfunction* which are of pivotal importance in the pathogenesis. In addition, ROS induce the migration of resident hepatic pro-fibrogenic cells, resulting in *fibrosis* [6 - 8].

EXTRAHEPATIC AND INTRAHEPATIC MECHANISMS IN NAFLD

According to Byrne *et al.'s* conception [9], ***extrahepatic and intrahepatic mechanisms*** contribute to the development of NAFLD and NASH. We first discuss these basic factors of the pathogenesis.

Extrahepatic Mechanisms

Regarding *the lipolysis and non-esterified ("free") fatty acids (FFAs),* it is generally accepted, that increased delivery of FFAs to the liver from peripheral (adipose) tissues is fundamental to the development of NAFLD. Approximately, 60% of fat deposited in hepatocytes is generated from adipose tissue sources. In subjects with **insulin resistance** (IR), there is a *failure of insulin mediated suppression of hormone-sensitive lipase (HSL) resulting in uncontrolled lipolysis in the adipose tissue and increased FFA delivery to the liver* [10].

Saturated fatty acids (SFAs) are capable of exacerbating IR at the insulin receptor level, due to translocation of the protein kinase C delta isoform (PKCdelta) from the cytosol to the membrane compartment leading to impaired insulin receptor substrate (IRS) / PI3K (phosphoinositide 3-kinase) activation. High dietary saturated fatty acids are associated with IR, NAFLD and cardiovascular (CV) risk [11].

*Polyunsaturated fatty acids (**n-3 PUFAs**)* in the diet have a protective role in the pathogenesis of NAFLD and may be a potential therapeutic target [9].

Sterol-regulatory-element binding protein 1c **(SREPB-1c**) is a transcription factor for *de novo* lipogenesis and is negatively regulated by **n-3 PUFAs**, thus the activities of key enzymes for FAA synthesis are down-regulated with decreased hepatic fat deposition. In addition, n-3 PUFAs negatively regulate the activity of a *glucose-responsive-transcription factor* (carbohydrate-responsive-eleme-t-binding protein, **ChREBP**), disrupting its translocation from the cytosol to the nucleus. High dietary intake of simple *sugars or fructose* increases *de novo* lipogenesis [12].

*Phosphatidylcholin*e **(PC)** consists of medium-chain saturated fatty acids, and it is essential for the synthesis of VLDL particles and for the incorporation of neutral lipids into these particles. PC deficiency is associated with hepatic fat deposition secondary to the impaired export of VLDL particles [13].

CHAPTER 6

Metabolic Diseases and NAFLD

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of elevated liver enzymes and chronic liver disease in the Western countries. NAFLD has been noted to be common in patients with obesity, hypertension, type 2 diabetes and atherogenic dyslipidemia. In the United States, it is estimated that NAFLD affects 20-30% of the general population. In patients with diabetes, the prevalence of NAFLD has been reported to be 70%. A high prevalence of NAFLD was found in patients with type 2 diabetes and obesity (90%). NAFLD is an independent predictor of future risk of cardiovascular diseases, and metabolic syndrome as well.

Keywords : Cardiovascular, Cholesterol, Diabetes, Dyslipidemia, HOMA-IR, Hypertension, Inflammation, Insulin resistance, Lipid disorder, Metabolic syndrome, Obesity, Triglyceride.

INTRODUCTION

NAFLD is one of the most prevalent hepatic diseases in the developed countries [1]. It affects approximately 20 to 30% of the population [2 - 4]. The prevalence of NAFLD, however, also depends on the ethnical group and on the presence of other diseases. It occurs in 5 to 40% of populations in Asian countries [5]. A study in the USA showed that NASH occurred at the highest rate among Hispanics (47%) and at the lowest rate in the black population (24%) [6].

The prevalence of NAFLD may even be 90% in obese people, and 70% in patients with diabetes [7, 8]. The results of two large European studies showed the prevalence of 42.6-69.5% for NAFLD in patients with type 2 diabetes mellitus [9, 10].

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Other metabolic diseases that constitute a part of the metabolic syndrome, such as hypertension or atherogenic dyslipidemia (hypercholesterolemia, hypertriglyceridemia, or a combination of these) can also be found more frequently, in 20 to 80% of patients with NAFLD [11].

In the developed countries cardiovascular (CV) diseases are the leading cause of mortality [12]. According to the report of the World Health Organization (WHO), 17.3 million people died as a consequence of CV diseases in 2008, which represented 30% of the total mortality in that year [13]. This number increased further to 2012, when 17.5 million people died due to CV diseases, representing already 31% of the total mortality [14].

Results published up to now showed that the presence of NAFLD increases the risk of both total mortality and mortality due to CV diseases [15 - 18].

On average, *NASH* occurs in approx. 2 to 3% of the population [19, 20]. A survey among patients with type 2 diabetes mellitus, however, showed a prevalence of 63-87% for NASH and 22-60% for moderate to severe fibrosis [21]. In the USA, NASH is currently the 3rd most common cause of liver transplantation after hepatitis C and alcoholic liver disease [22, 23]. It is estimated, however, that NASH may be the most frequent cause of liver transplantation between 2020 and 2025 [23].

OBESITY

Up to now, the results show a close correlation between obesity (mainly abdominal obesity due to increased visceral adipose tissue) and the elevated risk of developing and progressing NAFLD and NASH (Fig. **1**) [24 - 28]. Adipose tissue has been referred to for years as an endocrine organ that produces, among others, tissue-specific cytokines, so-called adipokines as well. These cytokines [*e.g.* adiponectin, leptin, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), resistin] may influence the extent of insulin sensitivity, the decrease of which shows a close correlation with the increase in the risk of developing NAFLD [29]. In addition, the increased amount of free fatty acids (FFAs) released from the adipose tissue also has a decisive importance in the progression of hepatic lesion [30].

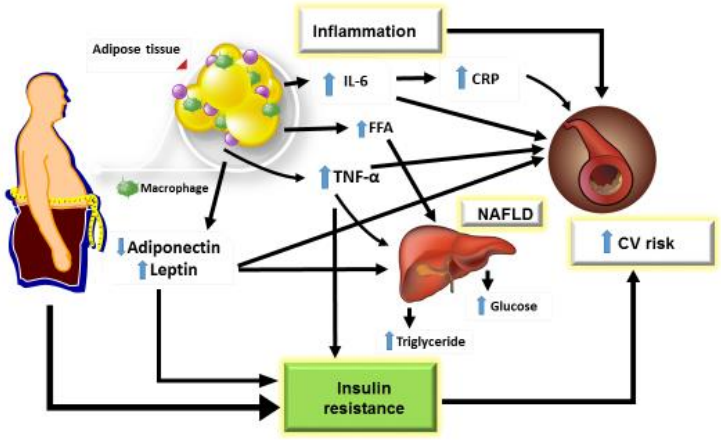

Fig. (1). Connection between abdominal obesity and NAFLD.

CRP = C-reactive protein; FFA = free fatty acids; IL = Interleukin; TNF-α = tumor necrosis factor alpha.

Several studies showed insulin resistance, *i.e.* an elevated value of homeostasis model assessment of insulin resistance (HOMA-IR) index exceeding 5.8 in non-diabetic overweight patients with NAFLD [31, 32].

In addition, results published up to now show a correlation between the diameter of adipocytes and the extent of hepatic lesion as well [33]. In the study of Wree *et al.* the data of 93 severely obese individuals (mean age 43 years; average BMI 52 kg/m^2) with NAFLD or NASH were compared before bariatric surgery and 6 weeks after the intervention. Also, a relationship has been demonstrated between increased adipocyte diameter and factors related to hepatic lesion such as lower adiponectin level, and higher levels of C-reactive protein (CRP), leptin, FFAs, transaminases and apolipoproteins [particularly apolipoprotein C-III (apo CIII)]. However, according to their results adipocyte cell diameter was found to be independent of BMI and gender as well, as demonstrated previously by O'Conell *et al.* [33, 34].

An approximately 5% reduction of body weight already mitigates the degree of steatosis and the concentrations of transaminases as well [35 - 37]. However, an approx. 10% reduction of body weight is often associated with a histological improvement and a moderated progression of hepatic impairment [35]. Nevertheless, a rapid reduction of body mass (24% in 8 weeks) may increase portal inflammation and the degree of fibrosis [38]. These results also

CHAPTER 7

Management of NAFLD

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Abstract: Nonalcoholic fatty liver disease and steatohepatitis are the most common chronic diseases of the liver. The process of their development has not yet been fully elucidated. It is characterized by insulin resistance, hepatic lipid accumulation with a secondary pathologic production of free radicals that induce inflammatory processes and fibrosis. There is no evidence-based therapy. It is important to eliminate the pathogenic factors (excess body weight, disordered carbohydrate metabolism, and hyperlipidemia). Potential modalities of a causal therapy include cannabinoid receptor 1 antagonists which do not cross the blood-brain barrier, cannabinoid receptor 2 agonists; selective serotonin 2C receptor agonist, thiazolidinediones, incretins, and dipeptidyl peptidase inhibitors. Additional therapeutic possibilities of the future may target antioxidant defense, immune-mediated mechanisms, apoptosis, and lipogenesis.

Keywords: Diabetes mellitus, Dipeptidyl peptidase inhibitors, Hyperlipidemia, Incretins, Insulin resistance, Lifestyle modification, Nonalcoholic steatohepatitis, Nonalcoholic steatosis, Thiazolidinediones, Treatment, Vitamin E, Weight loss.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an acquired hepatic disease that may be characterized, in addition to lipid accumulation, by lobular inflammation (nonalcoholic steatohepatitis, NASH), accruement of connective tissue (fibrosis) and fibrotic remodeling (cirrhosis) [1].

NAFLD is the most frequent chronic liver disease, which is the most common cause of hepatic dysfunction [2]. Depending on the method of screening, NAFLD may affect 20% population on average (6 to 33%), however, according to more recent data 30 to 50% of the population [1 - 6]. Its prevalence is increasing all over the world, unfortunately also among children [1, 7] due to the epidemic spread of obesity [1]. The long-term prognosis of NAFLD is good. The associated

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mortality is similar to that of the general population; however, it is also influenced by the accompanying diseases. The liver mortality of NASH is three times higher in comparison to the simple hepatic steatosis (8.6% *vs.* 1.7%) [1]. Its importance is further increased by the fact that it is a pathogenic factor of hepatocellular carcinoma [3, 8]. In patients with chronic HCV infection the prevalence of steatosis has been estimated to be about 55% [9, 10]. The viral steatosis (genotype 3) and metabolic (non-3 genotype) steatosis predict a more rapid progression of fibrosis, an increased risk of development of hepatocellular carcinoma, and a negative response rate to interferon-based treatment [9].

NAFLD may occur in patients of all ages, with an average of 47 to 53 years [2]. Its prevalence increases with the age; it is determined by genetic and environmental factors [1]. As for the involvement of genders, data of literature are equivocal. Certainly, the course of the disease is different in male and female patients [6]. Patients (69 to 100%) are obese (BMI >30 kg/m^2); they often have type 2 diabetes mellitus (34 to 75%) or hyperlipidemia (20 to 81%), and almost a half of them have hypertension [2].

The development of NAFLD and the process of its progression have not yet been fully elucidated. Its pathogenetic mechanism is characterized by the theory of 'double hits': 1) accumulation of triglycerides in the liver, and then 2) pathologic production of free radicals inducing inflammatory processes and fibrosis formation [2]. Increased uptake of fatty acids by the hepatocytes, decreased uptake of lipoproteins by the tissues, increased *de novo* hepatic lipogenesis, decreased hepatic output of triglycerides and decreased mitochondrial fatty acid oxidation may all contribute to hepatic steatosis [2, 11]. The development of *insulin resistance* is a key event in the development of fatty liver [11 - 15].

The accruement of fatty acids induces a secondary *release of free radicals* by activating the microsomal monooxygenase system, as well as by mitochondrial β-oxidation and peroxisomal ω-oxidation of the free fatty acids, which results in lipid peroxidation and a damage of proteins and DNA. Products of lipid peroxidation inhibit the delivery of triglycerides from the liver, thus making the steatosis more severe. Increased expression of tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β) lead to necrosis of hepatocytes and inflammation; these molecular events promote the transformation of stellate cells to collagen-producing myofibroblasts and the fibrogenesis. Free radical processes are also enhanced by portal endotoxemia, mitochondrial disorders, leptin production of the stellate cells, and moderate accruement of iron [12, 13, 16, 17] (Fig. **1**).

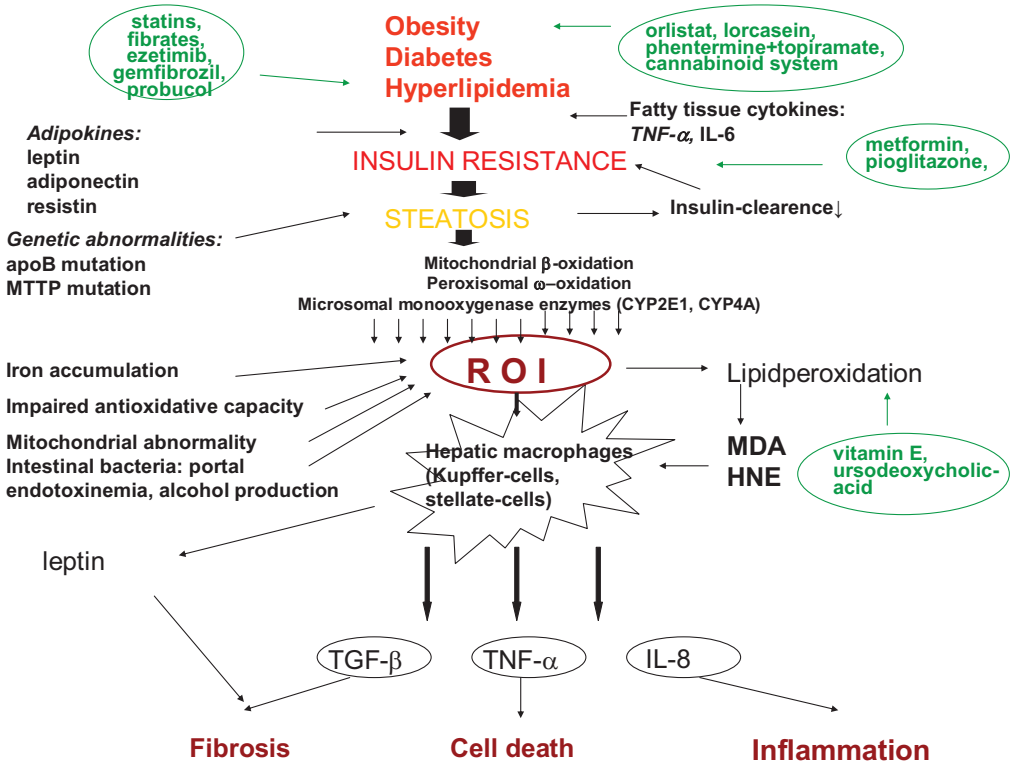

Fig. (1). Pathogenesis of NAFLD and NASH, and the possibilities of the therapy.

HNE=hydroxynonenal; IL-8= interleukin-8; MDA= malondialdehyde; MTTP= microsomal triglyceride transfer protein; ROI= reactive oxygen species; TGF-β= transforming growth factor-β; TNF-α=tumor necrosis factor-α

There is no known, evidence-based specific therapy. The management of patients with NAFLD includes treating of metabolic co-morbidities, such as obesity, hyperlipidemia, insulin resistance, and diabetes mellitus [3]. A second strategy is to prevent/reverse hepatic cellular damage induced by lipotoxicity. This can be achieved by inhibiting lipid peroxidation and oxidative stress, or through the use of anti-inflammatory, anti-apoptotic or other hepatoprotective agents. These two strategies may be combined at best [17 - 21].

Treatment

Reduction of Body Weight

Gradual reduction of body weight, together with concomitant use of diet and physical activity, improves insulin sensitivity and mitigates the histological

CHAPTER 8

Nonalcoholic Fatty Liver Disease and Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and the third leading cause of cancer death in the world. HCC has a poor prognosis unless recognized at an early stage, underscoring the importance of prevention. HCC most often develops in cirrhosis associated with chronic viral, toxic, or genetic liver injury. Notably, HCC has a rising incidence in developed societies with an increasing evidence for the role of nonalcoholic fatty liver disease (NAFLD), which has become the most common liver condition mirroring the spread of obesity and type II diabetes. A significant proportion of HCC associated with NAFLD may occur in the absence of advanced fibrosis or cirrhosis, posing a major challenge to cost-efficient risk stratification. Beyond the strong tumorigenic milieu of cirrhosis, molecular mechanisms of hepatocarcinogenesis in NAFLD include adipose tissue expansion with a pro-inflammatory adipokine profile, general and tissue-specific lipotoxicity, and the cell growth promoting effects of elevated insulin levels. Altered gut microbiota and microRNA deregulation may also contribute to HCC development in NAFLD. After reviewing these topics, the chapter provides a brief overview of the clinical characteristics, screening, and novel opportunities in the chemoprevention of NAFLD-related HCC.

Keywords: Adipose expansion, Cancer prevention, Cancer surveillance, Cirrhosis, Cryptogenic cirrhosis, Diabetes, Dysbiosis, Hepatocarcinogenesis, Hepatocellular carcinoma, Hyperinsulinemia, Insulin resistance, Lipotoxicity, microRNA, Nonalcoholic fatty liver disease, Nonalcoholic steatohepatitis, Noncirrhotic cancer, Obesity, Oncogenesis, Proinflammatory adipokines.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related

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mortality, accounting for more than 500,000 deaths per year worldwide [1]. The majority of HCC cases have been associated with cirrhosis due to chronic infection by hepatitis B virus (HBV) or hepatitis C virus (HCV) and toxic injury from excessive alcohol consumption [2]. HCC is one of the few major cancers becoming increasingly common in the US, as the age-adjusted incidence of HCC has grown from 1.5 to 4.9 per 100,000 individuals in the past 30 years [3]. This trend has been linked to the earlier spread of chronic hepatitis C in this country, but nonalcoholic fatty liver disease (NAFLD) is increasingly implicated in the rising HCC prevalence [4 - 6]. It is therefore critical to improve our understanding of the link between NAFLD and HCC.

NAFLD is strongly associated with obesity and related metabolic disorders such as type II diabetes, and it has become the most common liver condition in the US and other developed societies [7 - 10]. NAFLD is a spectrum of liver disorders, ranging from isolated steatosis to nonalcoholic steatohepatitis (NASH) [11, 12]. Within 10 years of duration, NASH is expected to progress into cirrhosis at a rate of 20% and may lead to major complications, such as portal hypertension, liver failure, and hepatocellular carcinoma (HCC) [11, 13]. The risk of HCC is substantial in advanced NAFLD and the yearly incidence of HCC may reach 2% to 4% in NAFLD-cirrhosis [14, 15], which exceeds the 1.5% incidence justifying HCC surveillance by current recommendations [16]. In a recent survey of the US Surveillance, Epidemiology and End Results (SEER) registry across a 6-year period (2004 to 2009), the number of NAFLD-HCC showed a 9% annual increase [17]. Moreover, a recent analysis of the United Network for Organ Sharing (UNOS) database found that the number of liver transplants for HCC related to NASH has quadrupled from 2002 to 2012 and advanced NAFLD is currently the second leading cause of HCC-related liver transplantation [18]. These data indicate an urgent need to find efficient ways of preventing, detecting, and managing the complications of NAFLD progression including the development of HCC. Now that the cure of chronic hepatitis C is within the reach due to the discovery of highly efficient direct acting antiviral agents, it has become imperative to focus on NAFLD as the next frontier in our efforts to halt the rising prevalence of HCC.

RISK FACTORS OF HCC IN NAFLD

Obesity and Diabetes

There is ample evidence that the risk of developing HCC is increased in obesity and associated metabolic disorders. In a large cohort of 900,000 American adults

participating the Cancer Prevention Study II, men with a body mass index (BMI) over 35 kg/m^2 died from liver cancer 4.5-fold more often than their non-obese counterparts [19]. Excess body weight increased the risk of liver cancer by 17% for overweight subjects and 89% for the obese in a meta-analysis of 11 cohort studies encompassing over 11,000 individuals [20]. Most of the 26 million Americans currently affected by diabetes have some form of NAFLD and it is estimated that 25% of them have NASH, indicating an increased risk of disease progression to HCC [21, 22]. Indeed, several large US population-based studies indicated that diabetes is an independent risk factor of HCC [23, 24]. In a prospective cohort study based on the computerized national VA database, data on 173,643 Veterans with diabetes and 650,620 Veterans without diabetes with over 10 years of follow-up indicate that diabetes doubled the risk of chronic liver disease and HCC [25]. According to a recent study on almost 7,000 elderly Americans diagnosed with HCC in the SEER-Medicare database, obesity and diabetes have the greatest population-attributable fraction of risk factors for HCC in both males (36.4%) and females (36.7%), which is consistent with the large prevalence of these metabolic conditions in developed societies [26]. Synergistic effects of obesity and diabetes may result in higher rates of HCC [27]. Accordingly, elimination of diabetes and obesity would have a tremendous impact on reducing the incidence of HCC and NAFLD-related mortality.

NAFLD with Advanced Fibrosis

Epidemiological and long-term follow-up studies indicate that HCC may complicate NAFLD-cirrhosis in the absence of viral, toxic, or genetic liver disease [13, 28]. A prospective US study conducted over a period of 10 years found that NAFLD-cirrhosis was complicated by HCC in 10 out of the 149 cases compared to HCV-cirrhosis in which 25 out of the 147 cases developed HCC [29]. In another analysis from the US, cumulative incidence of NAFLD-cirrhosis was 2.6% per year compared to 4.0% per year among patients with HCV cirrhosis [14]. A Japanese study conducted over 5 years found that HCC occurred in 11.3% of patients with NAFLD-cirrhosis compared to 30.5% of patients with HCV-cirrhosis [15]. However, 5-year survival and recurrence rates of HCC for these 2 different etiologies were very similar [30]. These data indicate that the burden of liver cancer in NAFLD-cirrhosis and in cirrhosis due to viral or toxic causes is comparable.

NAFLD without Advanced Fibrosis

Several lines of evidence indicate that HCC may complicate NAFLD in the absence of advanced fibrosis or established cirrhosis. A recent study of a large US

CHAPTER 9

Non-Alcoholic Fatty Liver Disease in the Pediatric Population

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Abstract: In the past decade, obesity has reached epidemic magnitude among children and adolescents, thus associated pathologic conditions are increasing simultaneously. These conditions include insulin resistance, type 2 diabetes, metabolic syndrome, cardiovascular diseases (CVD) and fatty liver disease (NAFLD).

NAFLD, previously thought to impact adults only, shares many of the same features of the metabolic syndrome, a highly atherogenic condition. This drew increased focus to study the role of NAFLD in relation to higher overall mortality and morbidity rates and increased prevalence of cardiovascular disease (CVD).

Insulin resistance is the pathophysiologic hallmark of NAFLD, the most common form of chronic liver disease in children in today's time. It is characterized by triglyceride accumulation with secondary free-radical production, which induces inflammatory processes and fibrosis due to numerous causes and complex mechanism.

Recent studies indicate that NAFLD has high prevalence in obese children, which has serious cosequences without treatment. Early intervention is utmost important when NAFLD is diagnosed, which should include early lifestyle modification (nutrition and physical activity, avoidance of smoking), however, no evidence based therapeutic approaches exist.

Keywords: Cardiovascular risk, Children, Insulin resistance, Nonalcoholic fatty liver disease, Obesity.

INTRODUCTION

Being obese or overweight in childhood increases the risk of being overweight or obese in later life. Similar to adult obesity, childhood obesity has been associated with a number of comorbidities [1].

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As obesity is a growing epidemy throughout the world, including Hungary, where nearly one third of children between the age of 6-12 years are overweight or obese, problems rising from this condition are becoming a great burden for the individual and for the society as well. A number of associated conditions, like metabolic syndrome, cardiovascular disease, type 2 diabetes (T2DM), fatty liver are observed already in childhood. Fatty liver is presently the most common liver disease in children [2, 3, 35].

Liver plays a key role in glucose homeostasis, as it receives and metabolizes the absorbed nutrients, stores or redistributes them according to the actual metabolic state of the person. It is important to understand that glucose metabolism is highly dependent on liver function, and the liver also plays an important role in the metabolism of drugs, produces inflammatory and vasoactive factors. Glucose overload increases fat accumulation in the liver.

By definition, nonalcoholic fatty liver disease (NAFLD) is the accumulation of triglyceride in the liver in more than 5% of hepatocytes with a reported prevalence of 3% to 10% in the general pediatric population and reaching a prevalence of 80% in obese/overweight children [4]. NAFLD can also be described as the metabolic disease of the liver [5], which can be seen quite early in children.

A number of causes can be found in the development of NAFLD, the leading cause being central obesity in childhood with insulin resistance, which in turn is the consequence of high-energy intake food and sedentary lifestyle. These associations are partly mediated by fat mass and fat quality.

EPIDEMIOLOGY, PATHOGENESIS

The realistic prevalence of NAFLD in the general population is estimated to be around 30%, even with the widely varying epidemiological data [6]. The prevalence rises further in adults with obesity (57-98%) and diabetes (69%) reflecting the strong association with metabolic syndrome [7, 8]. A life-cycle analysis showed a reduction of life expectancy with up to 7 years in adults with obesity [9].

It is estimated that 170 million children under 18 years old worldwide are overweight or obese, which is more than 20% of all children in many countries [15].

Insulin resistance is often accompanied by NAFLD/NASH, and plays a leading role in its pathophysiology [10, 11].

Those who had altered liver function as well, namely elevated ALT were shown to have a threefold risk for developing type 2 diabetes (T2DM) in a study, but the correlation disappeared after the multivariate regression analysis in one study, so authors stated that NAFLD was not an independent predictor [12]. Nevertheless in other studies, authors found that NAFLD was an independent predictor of T2DM and a strong risk factor for prediabetes [13, 14].

Comorbidities, as fatty liver is increased in this population. According to the follow-up study by Feldstein *et al.*, 4 out of the 66 children with NAFLD develop T2DM 4-11 years after the diagnosis. Moreover, during a 20-year long follow-up study, 2 children died and 2 underwent liver transplantation for cirrhosis, though formerly childhood fatty liver was considered a benign alteration [16]. Nevertheless there are only sporadic data regarding children till now.

Fatty liver classification is based on the severity of the liver status from simple steatosis (NAFLD) to steatosis with fibrosis, necrosis and inflammation (NASH). In the last year, alarming reports have been published about NAFLD in childhood, for this population tends to be overweight and obese due to excess food intake and sedentary lifestyle [17, 18]. NAFLD is often described as the metabolic syndrome of the liver that may start early in life with a generational transfer from mothers with high BMI to their offsprings [19]. Thus NAFLD is strongly associated not only with metabolic syndrome and type 2 diabetes, but also with cardiovascular disease. Prediabetes was reported to reach 85% in NAFLD patients [5].

Though the complete pathogenesis remains still unexplained and differs from adults, insulin resistance seems to be the main background, with other pathological conditions involved.

NAFLD is often described as a two-hit or multiple-hit model, where the first-hit involves triglyceride (TG) accumulation in hepatocytes exceeding 5% of cells, and liver damage as second -hit, or further hits, depending on the origin and extent of damage (fibrosis). That has been accepted in adults, however in children fibrosis did not correlate with insulin resistance. In children, the second phase is more variable and most often reversible. Fibrosis rarely occurs, even if it does, however, it does not correlate with insulin resistance. Genetic predisposition and environmental factors are the possible factors to act as second-hits in children.

Heterogenous genetic background also contributes to the evolution of children's fatty liver disease, *e.g.* chromosomal abnormalities, as Turner syndrome, Down syndrome, inherited metabolic syndromes (Prader-Willi, Angelman, Bardet-Biedl, Cohen, *etc.*), inherited metabolic diseases, like mitochondrial and fatty acid

SUBJECT INDEX