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REVIEW ARTICLE

Non-alcoholic fatty liver infiltration in children: an underdiagnosed evolving disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) constitutes the most common liver disease, one that is still underdiagnosed in pediatric populations (as well as in the general population), this due to the progressive increase in childhood obesity observed both in developed and developing countries during the last few decades. The pathophysiology of the disease has not been thoroughly clarified yet. The condition displays common pathways in adults and children; however, there are age-related differences. Unlike adults, children with NAFLD require extensive laboratory analysis, because underlying pathologies other than obesity may contribute to the evolution of the disease. Despite the presence of several serum markers and imaging techniques that contribute to NAFLD diagnosis, liver biopsy remains the gold standard diagnostic procedure. Early intervention and obesity prevention are mandatory, as NAFLD is reversible at an early stage. If left undiagnosed and untreated, NAFLD can progress to steatohepatitis (NASH) and subsequent liver failure, a potentially lethal complication. Of note, there are no treatment options when advanced liver fibrosis occurs. This review summarizes literature data on NAFLD in childhood indicating that this is an evolving disease and a significant component of the metabolic syndrome. Pediatricians should be aware of this entity, screening children at high risk and providing appropriate early management, in collaboration with pediatric subspecialists.

Keywords Non-alcoholic fatty liver disease · Obesity · Children

Introduction

The dramatic increase in the prevalence of pediatric and adolescent obesity during the last few decades has led to a rise in related comorbidities, such as hypertension, dyslipidemia, insulin resistance, type 2 diabetes mellitus, obstructive sleep apnea, and non-alcoholic fatty liver disease (NAFLD) [1, 2]. The latter comprises a wide spectrum of liver damage, from simple steatosis, which is considered benign and reversible, to more severe forms of the disease, such as non-alcoholic steatohepatitis (NASH), which can result in liver fibrosis, cirrhosis, hepatic failure, and predisposition to hepatocellular carcinoma development later in life [3, 4]. NAFLD is the most common liver disease in pediatric populations in developed and developing countries but still remains an underdiagnosed entity. NAFLD is a hepatic manifestation of the metabolic syndrome and has been associated with both hyperlipidemia and insulin resistance [5, 6]. The current review summarizes data on the pathogenesis, risk factors, and diagnostic work-up as well as the available preventive and treatment options for pediatric NAFLD.

Epidemiology

The rapidly growing pediatric obesity epidemic over the past several decades has led to the increased prevalence of NAFLD in children and adolescents, especially in Western countries [7, 8]. The prevalence of NAFLD in the general population of Western countries is about 20–30%, with 2–3% estimated to have NASH. More specifically in children, the overall prevalence of NAFLD is 3–10%, rising to 40–70% among obese children [9]. As the gold standard for the diagnosis of NAFLD is liver biopsy, the true prevalence of childhood NAFLD and NASH is still unknown. A study based on autopsies of 742

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children aged 2 to 19 years who died from trauma estimated that the prevalence of NAFLD, adjusted for age, gender, race, and ethnicity, was overall 9.6%, being lower in children aged 2–4 years (0.7%) and higher in adolescents (17.3%), with the highest prevalence observed in obese children (38%) and in children of Hispanic origin (11.8%) [10]. Another study also based on medico-legal autopsy reports of 265 children revealed that NAFLD was present in 4.2% of children aged 6 months–18 years old, and NASH in 0.3%, while 55.6% of children with NAFLD were obese [11]. Most clinical studies investigating the epidemiology of NAFLD are based on circulating markers, such as serum ALT, or ultrasound findings. These studies mainly estimate the prevalence of the disease only in obese subjects. In a prospective study from Australia, NAFLD was present in 15.2% of adolescents ($n = 995$) who participated in the Western Australian Pregnancy Cohort (Raine) Study [12]; dietary patterns in early adolescence were associated with an increased risk of NAFLD later in life. In European populations, a study in many obese subjects ($n =$ 16,390) from obesity centers in Germany, Austria, and Switzerland revealed that NAFLD was present in 11% of the sample investigated, predominantly in males [13]. In Asian populations, the prevalence of NAFLD is lower. It was reported to be 7.1% in Iranian, 3% in Indian, and almost 4.5% in Japanese children [14–16]. The differences in NAFLD prevalence observed in several ethnicities could be attributed both to genetic factors and to different dietary patterns [17]. Studies in a large cohort of individuals, regardless of obesity, are required in order to accurately determine the prevalence of NAFLD in different ethnicities. Furthermore, replacement of liver biopsy by another non-invasive test of equal validity, either imaging or serologic, is urgently needed. A non-invasive procedure would be of profound importance, not only to diagnose primary NAFLD but most importantly, to recognize exacerbation of this disease under different circumstances, with serial appreciation of the evolving pattern in the same persons.

Pathogenesis, genes, and risk factors for NAFLD

Pathogenesis

The exact pathophysiological mechanisms leading to fatty liver and progression to NASH are not yet well understood, but it is generally accepted that both genetic and epigenetic factors are involved [18]. The "two-hit" or "multiple-hit" theory was proposed by Day et al. over a decade ago to explain the development and progression of the disease [19]. The "first" hit is the accumulation of fat within the hepatocytes. The most important factor at this stage is obesity and insulin resistance, which are responsible for the increased uptake of free-fatty acids by the hepatocytes [20]. The second "hit" or other multiple additional "hits" are essential for progression of the disease to liver inflammation and subsequent fibrosis, in other words, transition from NAFLD to NASH. Oxidative stress is a major factor associated with liver damage; it is attributed to hypoxia, mitochondrial dysfunction, and lack of antioxidants, leading to the generation of reactive oxygen species (ROS) [20]. ROS are responsible for liver cell damage, either in a direct toxic way (death) or indirectly through apoptosis. Several cytokines are also implicated in progression of the disease to NASH.

There is a direct and statistically significant relationship between obesity, i.e., excessive accumulation of body fat, and NAFLD. Bearing this in mind, together with the fact that, as is today widely accepted, fat, or adipose tissue, is an active endocrine organ secreting several hormones and cytokines, it can be assumed that obesity-related adipose tissue inflammation promotes metabolic dysregulation. Meanwhile, adipose tissue adiponectin is an anti-inflammatory cytokine produced by white adipose tissue: it displays multiple functions, such as improvement of insulin sensitivity, inhibition of free fatty acid uptake, and increased lipid export from hepatic cells [21]. Its expression is decreased in NAFLD patients [22, 23], whereas TNF-a, a proinflammatory cytokine is increased [24]. The abovementioned "two-hit" theory has been applied both in obese children and in adults, although possibly there are differences in disease pathogenesis between adults and children. For instance, unlike in adults [25, 26], the role of central obesity in children with NAFLD has not yet been clearly demonstrated [27–29], while it is possible that in childhood, overall obesity is associated with NAFLD development. Furthermore, despite obesity being the most frequent factor for NAFLD and NASH in children, there are several other conditions that could predispose to the disease, especially in children younger than 10 years of age, as presented in Table 1.

Genes

The role of genes in the pathogenesis of NAFLD is supported by studies demonstrating that different ethnicities have different prevalence of the disease, by the fact that not all patients with NAFLD progress to NASH and, finally, by the fact that NAFLD displays some hereditability, as 18% of patients with NASH have a first degree relative suffering from NAFLD [29, 30]. This latter observation should, at least in part, be interpreted with caution, as close relatives tend to live in relatively similar conditions, notably with regard to dietary patterns, and to be exposed to approximately the same environmental influences. Several genes have been implicated in NAFLD pathogenesis at several stages. For instance, a single-nucleotide polymorphism (SNP) of the patatin-like phospholipase-3 (*PNPLA-3*) gene which encodes for adiponutrin expression, namely the G allele, has been

Table 1 Diseases associated with NAFLD in childhood/adolescence and that should be included in differential diagnosis, even in obese individuals

Diseases associated with fatty liver in children/adolescents	
General/systemic	Obesity/metabolic syndrome
	Anorexia nervosa
	Acute starvation/rapid weight loss
	Total parenteral nutrition
	Inflammatory bowel disease
Liver diseases	Hepatitis B, C
	Autoimmune hepatitis
Genetic diseases	Cystic fibrosis
	Prader–Willi syndrome
	Turner syndrome
Metabolic disorders	Wilson disease
	A1-antitrypsin deficiency
	Celiac disease
	Mitochondrial and peroxisomal defects of fatty liver oxidation
	Familial hyperlipoproteinemias
	Diabetes type 1
Drugs	Antiepileptic drugs
	HIV treatment drugs
	Cancer treatment drugs
Hormonal disturbances	Polycystic ovary syndrome
	Thyroid disorders
	Hypothalamic-pituitary disorders

associated with liver fat accumulation and liver enzyme increase [31], although in children, no association with liver histological severity has to date been confirmed [32]. This SNP displays the highest prevalence in Hispanic populations and the lowest in African-Americans [33, 34]. A study performed in 200 children and adolescents with NAFLD (58% obese and 32% overweight) showed that the *rs738409 I148M PNPLA3* polymorphism was the main determinant of steatosis severity, also linked with dietary components, such as sweetened beverage consumption [35]. Moreover, SNPs resulting in enhanced *IL-6* and *TNF-a* gene expression have also been associated with insulin resistance and NASH [23]. Lipins, proteins that promote fatty acid oxidation by acting as co-regulators of gene expression by DNA-bound transcription factors, have also been associated with liver steatosis [36]. *Lipin1 rs13412852* SNP is associated with severity of liver damage and progression of fibrosis in pediatric patients with histological NAFLD [37]. The transmembrane 6 superfamily member 2 (*TM6SF2*) gene, predominantly expressed in the liver and intestine, is associated with plasma triglyceride (TG) concentrations [38]. Carriers of the *TM6SF2 E167K* variant have fatty liver due to reduced secretion of very low-density lipoproteins. As a result, they have lower circulating lipids and reduced risk of myocardial infarction, while being more prone to progressive NASH [39]. On the other hand, SNPs of the *UGT1A1* gene that lead to increased levels of bilirubin (Gilbert's syndrome) are associated with decreased incidence of NAFLD [40, 41]. These genes involved in NAFLD pathogenesis are summarized in Table 2.

Risk factors

Several risk factors have been associated with NAFLD development in children [40, 42]. The role of BMI and obesity has been previously discussed. Age per se is a risk factor, as NAFLD is far more prevalent in adolescents, rare in children < 10 years old, while probably absent in those under 3 years of age, in whom the presence of NAFLD is debated and does not exist as a clinicopathological entity [10, 40]. Sex hormones and the frequently unhealthy dietary patterns of adolescents could explain the higher prevalence of NAFLD in this age group [12]. Boys are more often affected by the disease than girls at a ratio of 2:1, regardless of age and BMI [9, 43, 44], but females have a different metabolic profile to that of males; the latter have especially elevated cholesterol and serum LDL concentrations, which are associated with a greater NAFLD risk than in girls [45]. On the other hand, the impact of gender on the development of NASH has yet to be elucidated. Indicatively, in adults, the prevalence of advanced fibrosis is higher in post-menopausal women than in men of similar age, a finding that additionally highlights the role of the loss of estrogens which occurs in NASH progression [46]. Ethnicity

Table 2 Genes involved in NAFLD and NASH pathogenesis

Genes involved in NAFLD pathogenesis	
Genes influencing lipid metabolism	• Apolipoprotein C3 gene
	• Beta-adrenergic receptor gene
	• Phosphatidylethanolamine N-methyltransferase gene (PEMT)
	• Patatin-like phospholipase domain-containing 3 gene variant (PNPLA3)
Genes influencing insulin resistance	• Adiponectin gene
Genes influencing glucose metabolism	• Glucokinase gene
Genes affecting oxidative stress	• Fat oxidation genes
	• Manganese-dependent superoxide dismutase (MnSOD) gene (enzyme that regulates ROS generation in mitochondria)
	• Glutathione S-transferase isoforms genes (ROS generation)
	• Myeloperoxidase gene (ROS generation)
	• Microsomal epoxide hydrolase gene (ROS generation)
	• Human hemochromatosis protein-encoding gene
Genes influencing inflammation and immune response	• Tumor necrosis factor (TNF)-alpha gene
	•Transforming growing factor (TGF)-beta gene
	• Interleukin Il-6 gene
	• Interleukin Il-10 gene
Genes involved in apoptosis	• Tumor necrosis factor (TNF)-alpha gene
	• Bcl-2 protein family genes

is an important factor, since, as already mentioned, large epidemiological studies showed that NAFLD is more common in Hispanic individuals compared to Caucasians and African-Americans [47–49]. Dyslipidemia is another risk factor, and a large percentage of children (20–80% according to different studies) may present with hypercholesteremia and/or hypertriglyceridemia [50, 51]. Individuals with NAFLD and elevated triglycerides are more likely to develop NASH. There is a strong association between fatty liver and metabolic syndrome and insulin resistance and presence of type 2 diabetes mellitus [52]. Children with metabolic syndrome have a five-fold increased risk for fatty liver compared to normal individuals. Celiac disease is an independent risk factor for NAFLD regardless of metabolic syndrome, but clinicians should also be aware of the co-existence of atypical celiac disease in obese children with liver dysfunction [53].

Dietary habits, including consumption of sugar-sweetened beverages, which has increased fivefold since the 1950s, have been associated with metabolic syndrome and fatty liver [54]. The fact that approximately 75% of all foods and beverages contain sugar in several forms constitutes a major public health problem. Concerns exist regarding the role of fructose in the predisposition for fatty liver. Two reviews published in 2014 suggested that the overall strength of evidence from observational studies is insufficient to prove an association between a hypercaloric fructose diet and fatty liver in healthy adults [55, 56]. Studies on this topic generally display inconsistent findings and are usually small, of short duration (less than 4 weeks), or of poor quality. Despite the lack of strong evidence relating high fructose consumption to NAFLD induction in healthy individuals, in patients with NAFLD, daily fructose ingestion is associated with increased hepatic fibrosis [57]. In obese adolescents, the risk of NAFLD is lower in those with lower fructose consumption [58], and, inversely, children with NAFLD are more prone to fructose beverage consumption than healthy children [59]. Endotoxins released by gut bacteria after fructose consumption seem to play an important role in NAFLD. Serum endotoxin levels are higher in children with NAFLD than in healthy individuals, and their levels are associated with insulin resistance and circulating concentrations of inflammatory cytokines [60]. Regarding low-calorie sweeteners, such as aspartame, stevia, and tryptophan, and their association in the pathogenesis of NAFLD, the only studies that presently exist are in experimental models (rats) [61–63]. Aspartame, when used long term, has been associated with hyperglycemia and serum triglyceride elevation, while upregulation of leptin and downregulation of adiponectin have been observed [61]. On the other hand, stevia-derived compounds and tryptophan supplementation in mice diet seem to reduce hepatic steatosis [63]. The abovementioned research findings should also be further investigated in humans.

Regarding other contributing factors, breastfeeding is protective against the disease and progression to NASH, as it protects from later development of obesity [64]. Some studies demonstrate that low birth weight neonates and children who showed rapid catch-up-growth also have an increased risk for subsequent obesity and NAFLD [65]; however, it is possible

that accelerated infant weight gain during the first 3 months of life is a stronger risk factor for NAFLD than low birth weight [66]. Finally, obstructive sleep apnea is another risk factor for fatty liver disease, regardless of the presence of metabolic syndrome manifestations [67].

Clinical presentation and differential diagnosis of NAFLD

Most children with NAFLD are obese adolescents who are usually asymptomatic. Symptoms are inconsistent and may include vague pain at the right upper quadrant (indicative of NASH), malaise, and fatigue. On clinical examination, findings can be hepatomegaly (almost 50% of patients) and acanthosis nigricans, indicative of NASH and insulin resistance, respectively [40, 68]. Waist circumference is a valuable anthropometric parameter for NAFLD and NASH evaluation in children [40, 69] and was proposed to be used among other parameters (besides total bilirubin and total cholesterol) for the construction of a non-invasive prediction model for the progression from NAFLD to NASH [68]. Therefore, it is essential to construct charts for waist circumference according to ethnicity and age to be used in clinical practice [69].

Suspicion of NAFLD is raised when obese children present with elevated serum liver enzymes (mainly ALT and γ-GT) and/or increased hepatic echogenicity on liver ultrasound, and diagnosis is established when all other causes of fatty liver have been excluded. According to an ESPGHAN position paper [40], in all infants and children < 10 years of age, a detailed diagnostic laboratory evaluation is essential to exclude all other causes of fatty liver. In older children (> 10 years), NAFLD is most likely attributable to obesity; however, disorders with hepatic manifestations should always be suspected in children. The differential diagnosis of NAFLD in children is shown in Table 1. The suggested detailed laboratory evaluation of these children is displayed in Table 3. It should be stated with emphasis that pediatricians need to be aware of the metabolic disorders causing liver dysfunction in both children and adolescents, and that obesity with elevated serum transaminase levels should not be the only factor to justify making a diagnosis of NAFLD [40].

A problem in clinical practice is the evaluation of liver transaminases in children since the cutoff of serum ALT in pediatric populations has not yet been established. There is no consensus on defining the upper normal limits of serum ALT, which display gender differences [70]. Some investigators define as pathological an ALT value > 40 U/L [28, 71, 72], but the SAFETY study revealed that the sensitivity achieved by using as 95th percentile ALT levels of 25.8 U/L in boys and 22.1 U/L in girls is too low to detect liver diseases, such as NAFLD [70]. Pediatricians should be aware that (a) children with normal or mildly elevated ALT serum levels can present with fibrosis in liver biopsy and that ALT is not a reliable marker for NAFLD staging [73], (b) high serum levels of γGT are associated with advanced liver fibrosis in NAFLD patients [74], and (c) the AST:ALT ratio is generally > 1 and increases as hepatic fibrosis progresses [75]. Clinicians should be aware of cases of diverse etiology with hypertransaminasemia and/or hyperenzymemias or pseudotransaminasemias (macrotransaminasemia).

Table 3 Suggested work-up in children with suspicion of NAFLD. Detailed history, clinical examination, and specific investigations are mandatory (e.g., sweat test and metabolic work-up) in adolescents. In children <10 years of age, thorough investigation is recommended (guidelines of the ESPGHAN Hepatology Committee, published in 2012)

Laboratory work-up in children/adolescents with suspicion of NAFLD
• Total Blood Count
• Biochemical profile: ALT, AST, GGT, ALT/AST ratio, urea,creatinine, electrolytes, fasting glucose and insulin, cholesterol, triglycerides, LDL-cholesterol, HDL- cholesterol, lipoproteins, uric acid, ferritin
• Coagulation tests
• Glycosylated hemoglobin, glucose tolerance test
• Thyroid function (hypothyroidism)
• Serum copper, ceruloplasmin, 24-h urine copper (Wilson disease)
• Liver autoantibodies (autoimmune hepatitis)
• A1-antitrypsin levels (A1-antitrypsin deficiency)
• Viral hepatitis (B, C) antibodies
• Sweat test (cystic fibrosis)
• Anti-transglutaminase A and total IgA (celiac disease)
• Serum lactate, amino and organic acids, plasma free fatty acids, acylcarnitine profile (metabolic disorders)
• Creatine-phosphokinase (CPK) (muscular dystrophy)
• History of drug treatment

Liver biopsy

Nowadays, liver biopsy is considered the most reliable tool not only for the diagnosis of NAFLD, differentiating this entity from NASH, but also for exploring the degree of inflammation and fibrosis and ruling out co-existence of underlying diseases, such as autoimmune hepatitis. The main drawback is the invasiveness of the technique, not easily applied in pediatric populations, which might lead to severe complications, and always carrying the risk of inadequate sampling [40, 76].

Regarding histological findings from the liver biopsy, NAFLD is established when at least 5% of the hepatocytes present with micro- or macrovesicular steatosis without severe inflammation or fibrosis, and this criterion is identical for children and adults [77, 78]. This stage is the most benign one and is reversible when treated early. The histological pattern of NASH in children, which may differ from that in adults, is of two types: type 1 (adult type) and type 2 (pediatric type) [79]. Type 1 steatosis is characterized by ballooning degeneration and lobular inflammation, with or without perisinusoidal

inflammation. Type 2 steatosis displays portal inflammation, with or without portal fibrosis, but ballooning degeneration and perisinusoidal inflammation are absent. However, there is an overlap, and both histological types can be observed in some pediatric patients [77, 78]. NASH type 2 ranges from 29 to 51% in children with fatty liver, being more frequent in younger children, especially boys of Asian and Hispanic descent and in those with more severe obesity, than individuals with NASH type 1 and is associated with advanced fibrosis [80, 81]. It seems that these two histological types have different etiology, pathogenesis, and response to treatment.

Several grading systems have been proposed for the assessment of the severity of NASH according to the histological findings from liver biopsy, such as the Brunt score and the NASH Clinical Research Network scoring system [77, 82]. According to these scores, there are four stages of fibrosis. Since the NASH score does not include portal inflammation, a histological feature characteristic of pediatric NASH, a new score, namely, the PNHS (Pediatric NAFLD Histological Score), has been proposed. A strong correlation between the PNHS score and the presence of NASH has been demonstrated [83].

A major clinical question is the ideal timing for liver biopsy in children with NAFLD. According to an ESPGHAN position paper (2012), criteria used for the timing to perform liver biopsy in pediatric patients are those proposed by Roberts et al. [40, 83] (Table 4), who stress that all laboratory investigations should be conducted before a liver biopsy is performed [40, 84].

The limitations and risks of liver biopsy have led to an effort to identify non-invasive biomarkers, which will reliably assess liver fibrosis and will replace liver biopsy as the gold standard method for NASH diagnosis and staging. These markers are related to apoptosis, oxidative stress, hepatic inflammation, and hepatic fibrosis. An example of a serum apoptosis marker is the intracellular intermediate filament protein cytokeratin-18 (CK-18), which is highly expressed in NAFLD patients, both adults and children, especially in those with NASH [85–89]. Nevertheless, there are conflicting results regarding the utility of this biomarker, as it is also over-expressed in other liver diseases.

Oxidative stress markers are molecules involved in oxidation pathways in the mitochondria, which are involved in the hepatic damage observed in NAFLD. Studies in pediatric populations showed correlation of some of these proteins (e.g., hepatic malondialdehyde, carbonyls, and bilirubin,) with hepatic fibrosis [90]. A study in 109 obese children and adolescents could not demonstrate a relationship between obesity with or without liver steatosis and oxidative stress [91]. In contrast, variants of the *UGT1A1* gene expressing as high levels of bilirubin contribute to reduced risk of disease onset [92]. Hepatic inflammation serum markers are cytokines: low levels of adiponectin and high levels of TNF-alpha and leptin have been observed in pediatric patients with NASH [93, 94], but their utility in predicting hepatic fibrosis in obese subjects is in doubt [95].

As far as liver fibrosis markers are concerned, these are classified as non-specific and specific. Non-specific liver fibrosis markers in children are serum level AST/ALT > 1 (with low sensitivity), high γ-GT levels, and waist circumference [20, 68, 75]. Specific serum liver fibrosis markers panels have been suggested, such as FibroTest and the FIB4 index, but these panels have been tested mainly in adults [96, 97]. In children, proposed panels are (a) the pediatric NAFLD fibrosis index, which is based on age, waist circumference, and serum triglyceride concentrations, although longitudinal assessment is required for its implementation in clinical practice [83], and (b) the European Liver Fibrosis (ELF) panel that comprises hyaluronic acid, amino-terminal propeptide of type III collagen, and tissue inhibitor of metalloproteinase I, having high specificity and sensitivity compared with liver biopsy findings; however, this should be confirmed by further studies [98].

Table 4 Criteria for liver biopsy in children with NAFLD, as proposed by Roberts E. A. In all cases, extensive laboratory investigation comes first, and liver biopsy follows only after an expert's opinion

Criteria for liver biopsy in children with NAFLD
Children < 10 years old
Children > 10 years old with a family history of serious fatty liver disease
Hepatomegaly and/or splenomegaly at clinical examination
Persistent hypertransaminasaemia and/or insulin resistance
Suspicion of underlying diseases, e.g., Wilson disease
Cases of hypothalamic dysfunction, e.g., Prader-Willi syndrome

Imaging techniques

Several imaging techniques have been investigated, mainly in adults, for NAFLD screening, which have advantages and disadvantages regarding diagnostic accuracy, specificity, and sensitivity, cost, and availability, although none of them can reliably distinguish NAFLD from NASH.

The most common imaging technique used for NAFLD diagnosis is ultrasound due to its wide availability, low cost, safety (lack of radiation), and high sensitivity and specificity for detection of steatosis in liver parenchyma [99]. Portal hypertension is also easily assessed. Steatosis appears as a bright or hyperechoic lesion of the liver, but in order to avoid false-

positive interpretations, liver echogenicity should exceed that of the renal cortex and spleen, resembling that of the pancreas. Also, an attenuation of the ultrasound wave should be noted, while loss of definition of the diaphragm and poor delineation of the intrahepatic architecture should also be observed [100]. A study performed in 208 pediatric patients with biopsy-proven NAFLD demonstrated that ultrasound displays excellent accuracy in NAFLD diagnosis but cannot determine fibrosis stage [101]. Another limitation is that it is operator dependent, and therefore, the operator's experience is an important factor for diagnostic reliability.

Magnetic resonance imaging (MRI) is very often used for fatty liver assessment in adults. The MRI type widely applied in such cases is chemical shift gradient-echo (GRE) imaging with in-phase and opposed-phase acquisitions, and the quantification of hepatic fat in hepatocytes is achieved by assessing the degree of signal intensity loss [98]. The reported specificity and sensitivity for GRE is 100% and 81%, respectively [102]. A common disadvantage of ultrasound, MRI, and computed axial tomography is the fact that a low amount of liver fat, i.e., < 30% wet weight, is usually not detected [103]. MRI is very appealing for application in children with NAFLD, since it is non-invasive and not associated with X-ray radiation, though more studies comparing MRI findings with liver biopsy histopathological findings are necessary. Proton MR spectroscopy (MRS) is the most accurate magnetic resonance method for fatty liver assessment since it can detect steatosis grade 1 (5–30% of fat) [104]. On the other hand, this technique is very expensive, requires operator expertise, is not available in all imaging units, and does not generate anatomic images.

Another imaging method for liver fibrosis assessment is elastography, which can use either ultrasound or FibroScan, and whose principle is based on mechanical excitation and generation of images from the tissue response to the localized excitation [105]. It has, however, several limitations. In obese subjects (BMI > 28), quantification of liver fibrosis is difficult, and steatosis may be confused with fibrosis [40]. Furthermore, this imaging technique can detect only advanced fibrosis, but cannot differentiate fibrosis of intermediate stages [106]. MR elastography is a new and promising technique that could complement MRS for the estimation of the degree of liver steatosis, but further studies in both adults and children are needed to prove its superiority over other established methods [107].

Treatment of NAFLD

The optimal management of pediatric NAFLD is obesity prevention, as other treatment options in children are quite limited. Lifestyle interventions, such as a healthy diet and moderate exercise, are the only recommendations for the pediatric population so far [108, 109].

Weight reduction in children with NAFLD is mandatory because the reduction of free fatty acids load from food results in better peripheral glucose utilization [110]. Clinical studies in obese children revealed that even a minimal reduction in weight can lead to an improvement of serum transaminase levels and ultrasonographic findings [111, 112] as well as to the reduction of ROS generation and inflammation [113]. It is very important for pediatricians to be aware that early stages of NAFLD (steatosis only) are reversible and intervention at this stage is crucial. Reduction of dietary intake of saturated fat and fructose (contained in many soft drinks) and an increased intake of fibers and omega-3 fatty acids are recommended. Weight loss should be achieved gradually (500 g/week), as excessive and rapid weight loss can result in more severe liver damage. In younger children, whose growth is particularly important, weight loss is not always needed, and no further weight gain is recommended.

Physical activity is also important, and both aerobic and resistance exercise are required to reduce hepatic fat content [40]. The combination of a healthy diet intervention and an increase in physical activity can improve even the histological findings [114]. Lifestyle interventions should be followed, if possible, by all family members to achieve improved patient compliance, and psychological support may also be required. Pediatricians have a leading role in patient management along with other pediatric subspecialties (gastroenterologists and endocrinologists).

The use of pharmacologic agents in pediatric populations is still controversial [108, 109]. Drugs such as insulin sensitizers (metformin), cytoprotective agents (ursodeoxycholic acid), and hypolipidemic drugs have minimal effects, while the latter are not allowed in NASH [115–117]. Antioxidant agents, like vitamin E, are promising, but they do not display better efficacy than weight loss alone in NAFLD [115]. A study in 44 obese children and adolescents showed that antioxidant supplements used daily for 4 months had a significant impact on serum transaminase levels but did not reduce γGT and serum markers of inflammation [118].

Probiotics and prebiotics positively modulate gut microbiota and could be potential treatment modalities in NAFLD patients, although the data in pediatric populations are as yet sparse [109]. Very promising in pediatric patients are omega-3 long chain polyunsaturated fatty acids, present in several natural foods. A 6-month administration of omega-3 fatty acids in children resulted in an increase in insulin sensitivity and improvement of liver echo findings [119], while these agents were even shown to improve liver histology in pediatric NAFLD [120]. Recent findings indicate that a combination of pharmacologic agents, like docosahexaenoic acid, choline, and vitamin E, could provide better efficacy in children with steatohepatitis [121].

Finally, in adults, bariatric surgery for severe cases of NAFLD yielded good results as regards hepatic histology, this probably attributable to the significant weight loss. Studies in children are necessary to replicate these results, although bariatric surgery is generally not recommended in growing children and adolescents [122, 123].

Conclusions

NAFLD occurs in children and adolescents mainly because of childhood obesity and is associated with increased morbidity and mortality in adulthood. As there are no effective drugs to modify the natural course of NAFLD, which, if left untreated, may lead to irreversible liver damage and even hepatocellular carcinoma, the only effective management is prevention of childhood obesity from the very beginning. Efforts should be made to sensitize health care professionals and parents to avoid the devastating effects of childhood obesity through effective prevention and promotion of a healthy lifestyle, including a healthy diet and moderate physical activity.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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