Non-alcoholic Fatty Liver Disease in Children

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of paediatric liver disease. It is the hepatic component of the metabolic syndrome (MetS), being almost always associated with obesity and insulin resistance, and frequently with abnormal triglyceride and/or cholesterol levels, abnormal blood pressure and impaired glucose tolerance. Its increasing prevalence among children and adolescents has been attributed to the obesity epidemic and the modern western lifestyle, with excessive consumption of refined carbohydrates and saturated fats in combination with low levels of physical activity. Key questions need to be answered concerning the potential progression of NAFLD towards more severe forms of liver derangement, the worth of performing biopsies in children with suspected NAFLD and the role played by the disease in promoting and anticipating the onset of cardiovascular disease (CVD) at an unexpectedly early age. The clinical relevance of these questions is undoubted, as NAFLD may cause significantly increased morbidity and mortality in adulthood.

Keywords

Insulin resistance (IR), metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), paediatric obesity

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Since non-alcoholic fatty liver disease (NAFLD) was first described in children in the early 1980s by Moran and colleagues,[1] a number of case series have been reported. Awareness of the disease has been growing among healthcare providers in the last few years, and nowadays NAFLD represents the most common cause of chronic liver disease in youth. Its growing prevalence in westernised countries has been attributed to the obesity epidemic.[2–6] Indeed, NAFLD develops in the setting of obesity, insulin resistance and all the abnormalities that make up the metabolic syndrome (MetS). Thus, even though NAFLD is not part of the current definition of MetS, it can be considered a contributing factor.[7–9] The association between NAFLD and components of MetS is one of the challenges that healthcare providers have to face when dealing with the disease. Further challenges include making a diagnosis of NAFLD, defining its prevalence, understanding its significance in terms of prognosis of certain histological findings that characterise paediatric NAFLD compared with the form observed in adulthood and, finally, demonstrating the worth of performing biopsies in children with NAFLD.

Epidemiology

The overall paediatric prevalence of suspected fatty liver disease is reported to be high as 3% in the US and Asia.[10–12] This figure is derived from a few population-based studies that used alanine aminotrasferase (ALT) levels and/or ultrasound to categorise children with NAFLD. The prevalence of NAFLD is very likely to be underestimated; of note, no data are available on the incidence of the disease in this age group.

It is widely accepted that the disease is more common in males[2,13] of certain minorities[2,13] with a central distribution of fatness. Its prevalence seems to increase with age.[2,13] In the US, data from the National Health and Nutrition Examination Survey 1999–2004 (n=2,450 children, age range 12–18 years) found an elevated ALT in 75 adolescents (3%), with prevalence rising to 6% in overweight and to 10% in obese individuals.[10] A similar prevalence of abnormal ALT (3.2%) was reported from the 1998 Korean National Health and Nutrition Examination Survey, in which 1,543 subjects 10–19 years of age were evaluated.[11] However, in a case series of autopsies performed on more than 800 children of various bodyweights and ethnicities who died of unnatural causes at two to 19 years of age, the prevalence of histologically proven NAFLD was 9.6%.[13] The Child and Adolescent Trial for Cardiovascular Health (CATCH), a school-based trial that recruited third-graders from public elementary schools in California, Louisiana, Minnesota and Texas (n=2,575) and followed them until 12th grade, found a prevalence of unexplained elevated ALT of 23% among obese adolescents (n=127).[14] The CATCH trial found that the highest rate of elevated ALT occurred in Hispanic adolescents (36%), followed by non-Hispanic whites (22%) and blacks (14%). Compared with Hispanic, Asian and indigenous peoples of North and South America, African-Americans seem to be less exposed.[2,12,14,15]

Data on prevalence have also been obtained using ultrasound as the method of diagnosis. In north Japan, the prevalence of echogenic liver in a sample of normal-weight young individuals (n=810, age range four to 12 years) was 2.6%.[12] In obese individuals and those with multiple metabolic abnormalities, the prevalence of NAFLD increases substantially, as reported by two studies performed in Italian and Chinese children, respectively. The Italian study (n=375) reported a 42% prevalence of echogenic liver among pre-pubertal and pubertal

children,[16] while the Chinese study reported echogenic liver in 77% of 84 obese children and both echogenic liver and abnormal ALT in 24% of them.[17] Among children with type 2 diabetes (n=115), the prevalence of abnormal ALT was 42%.[18]

Males and females show a different prevalence of NAFLD, with the disease being more common among boys. Furthermore, boys also seem prone to presenting with a histological pattern of steatohepatitis, also known as type 2 non-alcoholic steatohepatitis (NASH), which is different from the histological features observed in adults (type 1 NASH). By contrast, girls more frequently have type 1 NASH. These differences in prevalence and histology have been ascribed to a permissive role for testosterone and a protective role for oestrogen.[19]

Clinical Aspects

The course of NAFLD may be completely asymptomatic, with the disease discovered after an accidental report of abnormal ALT or echogenic liver. Children occasionally complain of fatigue, malaise and vague aching right upper quadrant discomfort. Hepatomegaly is more frequently observed. Acanthosis nigricans is quite uncommon among Caucasian children. With growing recognition of the disease, evaluation of ALT and liver echogenicity is now commonly performed in all overweight and obese patients, particularly in those presenting with visceral adiposity, clustering metabolic abnormalities and disturbed carbohydrate metabolism.

Data from the Non-Alcoholic Steatohepatitis Clinical Research Network (NASH CNR), a prospective study involving eight centres across the US, demonstrated in a sample of 176 children (mean age 12.4 years; 77% male) that increasing aspartate aminotransferase (AST) and γ-glutamyltransferase levels were independently associated with increasing severity of steatohepatitis. Increasing AST level and white blood cell count and decreasing haematocrit were independently associated with increasing severity of fibrosis. Area under the receiver operator characteristic curve for a model with AST and alanine aminotransferase was 0.75 and 0.74 for distinguishing steatosis from more advanced forms of NASH and bridging fibrosis from lesser degrees of fibrosis, respectively.[20] Even though data from the NASH CNR have confirmed the importance of liver aminotransferases in the diagnosis of either simple or complicated fatty liver, it is important to highlight that normal ALT does not exclude, in the presence of echogenic liver, the occurrence of either simple fatty liver or NASH. At the time of liver biopsy, up to 20% of children and adolescents with echogenic liver and previously fluctuating ALT have normal ALT, despite the biopsy confirming liver necro-inflammation and even fibrosis.[21]

A possible diagnostic algorithm would be as follows: overweight and obese children of any age (but particularly after puberty) who have increased waist circumference (as estimated according to waist circumference percentile for age, sex and ethnicity) should be investigated for disturbed carbohydrate metabolism (i.e. insulin resistance and impaired glucose tolerance [IGT]), dyslipidaemia and hypertension. We recommend performing a hepatic sonography and measurement of liver biochemistries in all overweight and obese children, with monitoring of their course over time.[3] The presence of clustering metabolic abnormalities (i.e. visceral adiposity, IGT, dyslipidaemia and hypertension) all increase the risk of having complicated fatty liver disease,[9,22] and these patients may require a liver biopsy, especially if there is a persistent and significant increase in ALT over time and no amelioration of these features following lifestyle changes.

Diagnosis

Liver biopsy is the gold standard for diagnosis of NAFLD. It allows the suspected diagnosis of NAFLD to be confirmed, and also distinguishes simple fatty liver from its more severe forms, such as NASH, fibrosis and cirrhosis. NAFLD is characterised by large droplet fat accumulation within the hepatocytes, with fat expected to be present in at least 5% of the hepatocytes. When fat accumulates without inflammation, it is called simple steatosis. NASH implies the presence of inflammation, with subtle differences in histology between children and adults. NASH type 1, which is more common in adults, is characterised by an infiltrate of polymorphonuclear leukocytes, with inflammation and fibrosis typically located in the perivenular zone. Ballooning degeneration of hepatocytes and mallory hyaline are other typical findings. NASH type 2 is commonly described in children. It is characterised by a different pattern of inflammation (mononuclear inflammatory infiltrate, with periportal distribution) and, rarely, Mallory hyaline.[4,23–26] In this context, periportal damage may represent an early response of the hepatocytes to environmental insults,[4] or may depend on the individual's genetic background. In fact, when series of children with biopsy-proven NAFLD are compared, different distributions of type 2 and type 1 NASH and an overlap of both are observed. These populations differ in terms of ethnicity, degree of overweight and insulin resistance. In the San Diego series, type 2 NASH was described in 51 patients out of 100, type 1 in 17 subjects and an overlap of both types in 32 cases.[19] In the Rome sample,[27] only 2.4% of 84 patients met the criteria for type 1 NASH, and 29% met the criteria for type 2. The combination of both types was found in 52% of patients. Interestingly, the Non-Alcoholic Steatohepatitis Clinical Research Network has recently shown that portal chronic inflammation is associated with greater histological severity of the disease, including advanced stages of fibrosis, in children.[23] Despite being able to provide valuable information, liver biopsy does have significant limitations, including cost and risks. Thus, liver biopsy is not feasible in population-based studies and outside of tertiary healthcare centres.

Serum ALT and liver ultrasound are commonly used as alternative means of diagnosis, ideally in conjunction with negative markers for other types of liver disease.[3] The limited sensitivity and specificity of ALT in the diagnosis of NAFLD have been discussed above. These limitations can be overcome in part by coupling ALT with liver ultrasonography.[28] The basis of sonographic evaluation of fatty liver is that fat within the liver simultaneously scatters and attenuates the ultrasound beam. The scattering causes the liver to appear hyperechoic (or bright), and the attenuation causes progressively greater signal loss at greater depth from the skin surface. Thus, the presence of fat in the liver can be inferred if the liver is both hyperechoic and associated with depth-dependent signal reduction.[29,30] Studies in adults with NAFLD demonstrate that sensitivity falls when evaluating patients with less than 30–43% steatosis on biopsy,[28,31] while the accuracy of ultrasound for liver fat detection in children has not yet been established. A major limitation is the inability to discriminate between simple steatosis and steatohepatitis. Furthermore, the technique is operator- and machine-dependent, its results are not reproducible and the results are influenced by confounding variables, including body habitus[32] and co-existing fibrosis and inflammation.[29,31,32]

An interesting modality for the assessment of NAFLD is magnetic resonance imaging (MRI), which allows the severity of liver fat to be graded;[33] however, its use seems to be limited to research investigations. The most commonly used MR technique in assessment of liver fat is phase-shift imaging.[33] This method is rapid (data can be acquired in a single breath-hold), reproducible, operator-independent and widely available on routine clinical scanners.[33–35] Its major limitation is that it incorrectly assumes that the signals from fat and water are directly proportional to the amounts of fat and water.[36]

In the search for non-invasive imaging methods for screening, diagnosis and longitudinal assessment of patients, the use of transient elastography has begun to receive increasing attention. Very recently, a study demonstrated that this technique is accurate in discriminating children without fibrosis from those presenting with significant/advanced fibrosis, but it fails to identify children with mild forms of fibrosis.[37] Thus, there is still a need to develop a safe, inexpensive and reproducible method of evaluating hepatic fat content, fibrosis and inflammation.

Non-alcoholic Fatty Liver Disease and Cardiovascular Disease

Over-nutrition and poor physical activity, which characterise the westernised lifestyle, cause fats to accumulate largely in adipose tissue and, inappropriately, in muscle and liver. This concept has been simply described as the 'overflow hypothesis'.[38] Ectopic accumulation of fats as triglycerides within myocytes, hepatocytes and adipocytes is associated with local and systemic insulin resistance. The time-course relationship between hepatic and systemic insulin resistance is a 'chicken and egg' question that has still not been answered. Undoubtedly, both gluconeogenesis and *de novo* lipogenesis are enhanced in NAFLD as a result of hepatic insulin resistance. Simultaneously, because of the systemic insulin resistance, lipolysis is abnormally suppressed. The decrease in the hepatic rate of lipid export contributes to the abnormalities in blood lipids observed in these patients. Thus, hypertriglyceridemia, low high-density lipoprotein (HDL) concentrations and small dense low-density lipoprotein (LDL) particles are common abnormalities. The interactions between hepatic and systemic insulin resistance and fat metabolism have been mainly studied in adult patients and are extensively described elsewhere (for a review on the role played by insulin resistance in the pathogenesis of NAFLD, see reference 39). These concepts deserve an exhaustive discussion, which is far from the intention of this brief article. Conversely, what we would like to address are the recent advances in pathogenesis deriving from evidence in paediatric settings that suggests a key role of NAFLD in the development and progression of cardiovascular disease.

Elevated triglycerides have been found to be associated with hepatic steatosis in various series of children.[17,40] Hepatic fat content, measured using fast MRI, in 49 obese adolescents with normal glucose tolerance was associated with a pronounced dyslipidaemic profile characterised by large very-low-density lipoprotein (VLDL), small dense LDL and decreased large HDL concentrations.[41] The same authors in a later study observed a significant association between intra-hepatic fat content and higher levels of two-hour plasma glucose after an oral glucose tolerance test.[42] Via unknown mechanisms, intra-hepatic fat content may mediate cardiovascular risk. Otherwise, a more pronounced pro-atherogenic profile, impaired carbohydrate metabolism and an imbalance between pro-inflammatory (i.e. tumour necrosis factor [TNF]-α[43]) and anti-inflammatory molecules (i.e. adiponectin[44]) may contribute to enhancing the cardiovascular risk of patients with NAFLD.

Recent investigations have demonstrated that obese children with NAFLD have an increased carotid artery intima media thickness, a marker of early endothelium dysfunction, compared with obese children with normal ALT and no echogenic liver.[45,46] In contrast to these results, no enhanced cardiovascular disease, as estimated by means of carotid intima media thickness (Manco and Nobili, unpublished data) or 24-hour urine albumin excretion,[47] was observed in children with biopsy-proven NAFLD compared with obese controls.

A limition of all of these studies that have tried to address the association between NAFLD and cardiovascular risk is the strong association between the hepatic and the visceral content of fat.[48] Again, we face a chicken and egg question since we cannot demonstrate which comes first, hepatic or visceral fat. Whatever the answer to this question is, NAFLD can no longer be considered a benign disease.

It seems that the focus of researchers' attention should be shifted from the muscle–adipose tissue axis to the cross-talk between the liver and visceral fat tissue. Direct liver injury or intra-hepatic immune responses are the usual culprits that initiate and perpetuate liver damage caused by anything from alcohol to viruses. The evidence suggests that the interaction between the adipose tissue and the innate immune system that occurs in obesity may be responsible for producing the low-grade inflammatory state that accompanies the metabolic syndrome. Of note, the Toll-like receptors (TLRs), notably TLR4, are located in both the liver and the adipose tissue. Insulin resistance and innate immune response are ancestral mechanisms of defence against pathogens, including nutrients. Activation of both systems can be mediated by TLRs.[49] In this context, endotoxin, otherwise known as lipopolysaccharide (LPS), a product derived from Gram-negative bacteria, can be a trigger common to both pathways. Recent evidence suggests a close and significant association of LPS with obesity,[50] NAFLD[51] and cardiovascular risk.[52] Data in children partially support these concepts. We have recently observed higher circulating levels of LPS in children with biopsy-proven NAFLD compared with children with an NAFLD activity score (NAS) >5.[53]

Treatment

Case series and controlled and uncontrolled trials have demonstrated the efficacy of lifestyle changes (achieved by a hypocaloric diet and physical exercise) in improving or normalising transaminases, the appearance of the liver (as revealed by ultrasonography),[40,54,55] the grade of steatosis and inflammation.[56,57] Lifestyle changes are not able to revert and ameliorate fibrosis.[56,57] After being evaluated for safety and following preliminary results from a couple of pilot studies,[58,59] the use of metformin or antioxidants has been compared with lifestyle change in controlled studies. Metformin 1.5g/day was administered in 27 overweight/obese young patients with biopsy-proven NAFLD (age nine to 18 years). The study was planned to last 12 months, with the aim of estimating the effect of metformin on liver enzymes. At the end of this period, the code was opened and the patients were asked to continue the treatment in an open fashion to estimate outcomes on liver histology. The control group (n=30) was obtained from a separate but parallel study that had identical inclusion criteria on the use of antioxidants in NAFLD. ALT significantly improved

from baseline with decreasing bodyweight in both groups. Steatosis was reduced in the two groups, as were ballooning and necro-inflammation. No significant changes in fibrosis were detected.[56]

In the controlled trial with the use of vitamin E, a total of 53 patients (age 5.7–18.8 years) were included. Patients were concomitantly randomised to alpha-tocopherol 600IU/day plus ascorbic acid 500mg/day (n=25) or placebo (n=28), and were treated for 24 months. The primary end-point of this study was the change in liver histology on repeated biopsy at 24 months. As well as for the metformin study, a significant improvement was seen in the grade of steatosis, lobular inflammation and hepatocyte ballooning and in the NAS in both groups. Levels of aminotransferases, triglycerides, cholesterol, fasting glucose and insulin, and also insulin sensitivity indices, improved significantly as well.[57]

In both studies,[56,57] lifestyle intervention consisted of a diet tailored to the patient's calorie needs and increased physical activity. It was able to induce a significant weight loss and a significant improvement in liver histology (except for fibrosis) and laboratory abnormalities in paediatric NAFLD. Neither metformin nor antioxidants performed better than lifestyle intervention alone.

Ursodeoxycholic acid (UDCA) is a cytoprotective agent that has been studied as a potential therapy in paediatric NAFLD. An Italian study[60] evaluated the efficacy of UDCA in 31 obese children (age four to 14 years) with abnormal serum aminotransferase levels. The children were assigned to the treatment according to their anticipated success with lifestyle modification. Children were treated with UDCA 10–12.5mg/kg/day with or without weight-reduction diet in comparison with diet alone or no intervention. At six months, the addition of UDCA to diet was no more effective than diet alone in reducing serum aminotransferases or the appearance of steatosis, as revealed by ultrasonography. No difference was observed between children treated with UDCA and those receiving no intervention; however, the children assigned to these treatment arms were those who were judged unlikely to comply with lifestyle advice.

Conclusion

NAFLD can no longer be considered a benign entity. No matter what the mechanisms of the association between NAFLD and cardiovascular risk are, counteracting NAFLD also means reducing or at least delaying the onset of cardiovascular disease. Studies on the pathogenesis of NAFLD in paediatric settings are urgently needed in order to enable efficacious treatment. The interaction among the gut, insulin-responsive organs and the liver can represent a potential target. ■

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