Review article

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PAEDIATRIC NON–ALCOHOLIC FATTY LIVER DISEASE: AN EMERGING THREAT

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The aim of this study was to review current knowledge about non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) and increase awareness among general paediatricians and family doctors. NAFLD and NASH represent the commonest causes of chronic liver disease in children at the present time. Increasing prevalence is thought to result from a combination of several environmental factors including high calorie intake, a carbohydrate-rich diet and a sedentary lifestyle. Frequent familial clustering indicates that some genetic factors, such as insulin resistance and different ethnic prevalence, could also have a role in pathogenesis. It is likely that NAFLD represents the initial step in pathogenesis step, whereas NASH is a more advanced, severe phase of the same process. Histopathologically, the main difference between the two is presence of an inflammatory component in NASH which may be lobular, portal or both, in addition to varying degrees of fibrosis. Due to relatively slowly progressive nature, this potentially preventable disease is likely to become the leading indication for liver transplantation in adulthood. The commonest phenotype is associated with truncal (central) obesity, which is also a well-recognised risk factor for chronic metabolic, cardiovascular, musculoskeletal and mental health disorders. Therefore, controlling excessive weight is an important public health measure, but due to its complex pathogenic background its implementation remains difficult. Regular physical exercise should be encouraged. Insulin sensitising agents, anti-oxidants, polyunsaturated fatty acids and probiotics have all been trialled as potential therapeutic agents but a favourable response is not universal. **Conclusion.** NAFLD is a common but difficult-to-treat cause of chronic liver disease. The management is complex and includes combination of promoting active lifestyle, calorie-restricted diet and weight loss. Medical treatment has a limited role.

Key words: Non-alcoholic fatty liver disease ▪ Non-alcoholic steatohepatitis ▪ Obesity ▪ Insulin resistance ▪ Liver transplantation.

Introduction

There is a growing concern about the rapid rise of non-alcoholic fatty liver disease (NAFLD) in children, closely associated with the exponential increase in global incidence of obesity(1). As the commonest present cause of chronic liver disease in paediatrics, NAFLD is threatening to become the leading indication for liver transplantation (LT) in adults after 2020 (2). This would potentially overstretch already overburdened LT services by a condition which is potentially preventable.

NAFLD is characterised by fat accumulation in the liver (steatosis) which may be accompanied by inflammation (steatohepatitis) and fibrosis. This process is likely to progress if the pathogenic mechanism is ongoing. Though the need for LT in the paediatric age is uncommon, the fact that the children as young as 8 years old may demonstrate significant fibrosis due to NAFLD represents a major concern for both liver disease and public health services. NAFLD was first described in children in 1983, three years after it was recognised in adults (3). There are many unanswered questions in paediatric NAFLD, including the natural history, histological diversity from the adult disease, and the optimal management. The term NAFLD encompasses a range of severity of disease from simple steatosis to non-alcoholic steatohepatitis, characterised by inflammation (lobular, portal or both) and fibrosis (4). The true prevalence of NAFLD is difficult to determine as the diagnosis is a histological one. Proxy markers of fat accumulation (echogenicity on ultrasound) and liver injury (elevated transaminases) in the absence of any other diagnosis have been used to estimate prevalence in population studies. The sensitivity, specificity and predictive value of these proxy markers are unknown and there are several reports of NAFLD associated with an elevation of transaminases in only 60% of cases (5, 6).

The NHANES study reported a prevalence of elevated ALT in the absence of other diagnoses of 8% in 5586 adolescents aged 12–19 years (7). Park et al. reported a 3.2% prevalence of NAFLD in 1543 Korean teenagers using ALT >40 IU/L as a cut off (8). In Japan an ultrasonographic population-based study found 2.6% of children to have NAFLD (9). The prevalence increases dramatically in obese populations – a study from Italy of 268 obese children demonstrated that 44% had NAFLD using ultrasound (10). Autopsy studies give a more accurate reflection of prevalence based on histological criteria. A report from San Diego of almost 800 children who died an unnatural death described fatty liver in 9.3% of all under 18 years, with non-alcoholic steatohepatitis (NASH) diagnosed in 3% (11).

The aim of this study was to review current knowledge about NAFLD and NASH and increase awareness among general paediatricians and family doctors.

Risk factors

The prevalence of NAFLD increases with age, and some studies have found a male preponderance (11-13). Ethnic variations also exist; Hispanic and Native American children are at higher risk compared to the Caucasians. Of note, despite a higher incidence of insulin resistance (IR), which is closely associated with the disease, black non-Hispanic children are less susceptible to NAFLD (11, 13, 14). Both genetic and environmental factors are likely to be involved in the ethnic distribution. Familial clustering is also commonly seen (15, 16).

Genome-wide association studies (GWAS) have demonstrated that single nucleotide polymorphisms (SNPs) may be associated with the distribution of NAFLD; *PNPLA3* (the gene for adiponutrin, an insulin regulated phospholipase) has been shown to be associated with both steatosis and inflammatory change/fibrosis in the liver biopsy (17). Two recent studies of paediatric cohorts have demonstrated that those with a variant in PNPLA3 (rs738409), are more likely to have NAFLD but not insulin resistance (18, 19). Other polymorphisms have been associated with the risk of developing steatohepatitis/fibrosis, including SNPs in IL-6 (174G/C) (20), tumour-necrosis factor-alpha (21) and Kruppel – like factor (KLF6) (22).

Nutrition and physical activity are important environmental factors determining risk of NAFLD, with lifestyle modification the primary recommendation in the clinical management of NAFLD (23, 24). Excessive caloric intake, sedentary lifestyle and lack of exercise contribute to weight gain which has been shown to contribute to the progression of liver fibrosis in NAFLD patients (25).

Specific dietary factors may also play either protective or antagonistic roles in the development and progression of NAFLD. Dietary analyses have suggested that higher meat and soft drink intakes (26), higher fructose consumption (27, 28) and higher consumptions of low-nutrient, high calorie, high salt food (29) are associated with NAFLD.

In addition, a lower consumption of polyunsaturated fatty acids, fibre and vitamins C and E have been associated with the development of NAFLD (30). A study of 930 Australian youths reported that the intake of a western diet at the age of 14 predisposed to developing steatosis on ultrasound at the age of 17 (31). Vitamin D has also been suggested to have a potential pathogenic role (32).

Pathophysiology

NAFLD is the hepatic manifestation of the metabolic syndrome (linking obesity, IR, hypertension and hyperlipidemia). IR is a finding in up to 80% of children with NAFLD, similarly to adults with the condition (14, 33, 34). The pathogenesis of NAFLD is still incompletely understood. The 'two hit hypothesis' proposed in 1998 (35) suggests that the imbalance of import versus export of fat into the hepatocyte initiates steatosis, which makes the cell more vulnerable to injury. A second hit in the form of oxidative stress damage, for example, is then required for progression to steatohepatitis and fibrosis. A more recent hypothesis suggests that fat accumulation in the liver is a protective mechanism, packaging away potentially toxic free fatty acids as triglycerides. However, when the capacity of the cell to accumulate triglycerides is overwhelmed, the tissue damage - steatohepatitis ensues (36). It is widely accepted that IR and the resulting hyperinsulinemia seem to play a major role in the development of hepatic steatosis and, likely, steatohepatitis. However, the molecular mechanisms leading to IR and its involvement in development of NAFLD are complex and have not yet been fully elucidated.

Hyperinsulinism

Hyperinsulinism results in increased delivery of lipids to the hepatocyte, increased de novo lipogenesis and decreased export via VLDL and decreased oxidation, all resulting in the production and release of toxic free fatty acids into the circulation.

Oxidative stress

Oxidative stress is also implicated in the pathophysiology of NAFLD (37). The mitochondria are overwhelmed by free fatty acids requiring oxidation and may not function as effectively. There is an increase in reactive oxygen species which results in damage to the hepatocytes.

Inflammation

NAFLD is closely associated with central or visceral obesity characterised by an increased waist circumference. This is well known to be a chronic inflammatory state predisposing to ischemic heart disease and cancer (38). Adipocytokines produced by adipocytes and infiltrating macrophages, are mediators of this inflammation. Increased production of adipokines such as leptin, visfatin and decreased production of adiponectin have been found in patients with NAFLD (39, 40).

Apoptosis

The principle mechanism of cell death in NAFLD is apoptosis which may be intrinsically or extrinsically mediated and in the liver may give way to an inflammatory response (41).

Fibrosis

The final common pathway of inflammation, oxidative stress and apoptosis is fibrosis; the accumulation of excessive extracellular matrix in the liver parenchyma with distortion of the architecture. Classic complications of chronic liver disease such as impaired synthetic function, portal hypertension and HCC may follow as a consequence. Therefore, more information about pathophysiology of liver injury in NAFLD may lead to better understanding of chronic liver injury, irrespective of the cause.

Diagnosis and histology

Children with NAFLD are often asymptomatic and incidentally identified during screening for obesity, or may present with non-specific abdominal pain or fatigue. The majority of children are overweight or obese and a true diagnosis of NAFLD in a child of normal weight should only be made after careful consideration of alternative causes such as Wilson disease and lysosomal acid lipase (LAL) deficiency (Table 1).

Table 1 Differential diagnosis of NAFLD

Clinical condition	Clinical / Biochemical features
Wilson disease	Low serum caeruloplasmin, high urinary copper pre- and post-penicillamine, high liver copper, Kaiser-Fleischer rings, mutational analysis may be informative
Alpha 1 antitrypsin deficiency	Alpha-1 anti-trypsin phenotype PiZ homozygosity
Drugs – steroids, amiodarone, alcohol, methotrexate, ecstasy, L-asparaginase, vitamin E, valproate, tamoxifen, antiretrovirals	History
Cystic fibrosis associated liver disease	Positive sweat test / mutational analysis
Malnutrition	
Coeliac disease	Tissue trans-glutaminase positive / positive jejunal biopsies
Hepatitis C	Positive serology
Parenteral nutrition associated liver disease	
Mitochondrial disease / fatty acid oxidase deficiency	Abnormal respiratory chain enzymes liver/muscle, abnormal acyl-carnitine, skin fibroblast studies, tissue mitochondrial depletion demonstrated, mutational analysis for nuclear genes
Metabolic disease: Lysosomal acid lipase deficiency (Cholesterol Ester Storage Disease)	Positive blood spot enzymology
Galactosaemia	Abnormal Gal-1-PUT serum levels
Fructosaemia	Reduced enzyme activity in liver biopsy, mutational analysis
Glycogen storage disease	Glycogen, not fat in liver biopsy, reduced enzyme activity in liver biopsy, mutational analysis
Peroxisomal disorders	Abnormal Very Long Chain Fatty Acids
Mauriac syndrome	Glycogen in liver biopsy
Hypobetalipoproteinaemia / abetalipoproteinaemia	Low or absent serum Apo1B levels
Lipodystrophies	Mutational analysis
Schwachman syndrome	Mutational analysis

Mild hepatomegaly and splenomegaly may be present on examination. Acanthosis nigricans (black pigmentation of axilla, skinfolds and neck) - often seen in children with IR, is found in 30% to 50% of those with NAFLD. The majority of children with NAFLD have IR as measured by homeostasis model assessment-insulin resistance (HOMA-IR: [fasting insulin (μIU/mL)])×[fasting glucose (mmol/L)]/22.5); insulin resistance is defined as HOMA-IR >2 (14). In the diagnostic work-up of NAFLD, alternative causes of chronic liver disease should be excluded including chronic hepatitis B and C infection, Wilson disease, α1-antitrypsin deficiency, autoimmune liver disease and drug toxicity (42). With an estimated 25% of the paediatric population classified as overweight or obese, the consideration of other treatable liver diseases such as Wilson disease in an otherwise 'incidentally' overweight child is essential. Conditions such as cystic fibrosis, malnutrition and parenteral nutrition associated liver disease may also present with a fatty liver on ultrasound and should be excluded on clinical or biochemical grounds. In addition, mitochondrial/metabolic disease and cholesterol ester storage disease may also look very similar on liver biopsy. Table 1 gives differential diagnosis for NAFLD.

Liver biopsy is the gold standard for diagnosis of NAFLD and also for differentiation of steatosis without inflammation from steatohepatitis and fibrosis. However, given the estimated prevalence of NAFLD, this is not always practical and at present the liver biopsy is often limited to those in whom an alternative diagnosis is suspected, those with evidence of more significant liver disease (transaminases greater than twice the upper limit of normal, or splenomegaly) and those participating in clinical trials. Definite diagnosis of NASH is based on a specific pattern of histopathological findings including macrovesicular steatosis, mixed or polymorphonuclear portal inflammation, ballooning degeneration of hepatocytes and fibrosis. In adults, the pattern of inflammation is largely lobular and fibrosis perivenular (Type 1 NASH), whereas in children periportal disease is common (type 2 NASH). The reason for this difference in histological pattern is not clear (43, 44)

Natural history and management

The natural history of paediatric NAFLD is not yet well defined. A number of reports in adult patients have demonstrated that the progression varies according to histological severity. Those with simple steatosis rarely go on to develop significant liver disease or die a liver-related death, although the mortality from cardiovascular disease is higher than that of controls. In a systematic review of 40 studies the odds ratio for mortality in those with NASH was 1.8 times that in controls. The liver-related death rate was between 11% to 17% in those with NASH (45). The paediatric studies are scarce and not controlled, but Feldstein et al. report follow up over 20 years with fibrosis progression in 4 of 5 re-biopsied patients and the need for transplantation in 2 teenagers with cirrhosis secondary to NAFLD (46).

Management

The mainstay of management of NAFLD in both children and adults is lifestyle change and in particular, a healthy diet and exercise. Several case series and uncontrolled trials have demonstrated the effect of weight loss on improvement of NAFLD (47-49). Eighty-four children (3-18 years) with NAFLD demonstrated a significant decrease in BMI, serum levels of fasting glucose, insulin, lipids, transaminases and liver echogenicity on US following a 12 month program of lifestyle advice consisting of diet and physical exercise (50). Fifty-three children comparing lifestyle intervention plus

antioxidant with lifestyle intervention plus placebo demonstrated similar improvements in both groups in terms of steatosis, inflammation, ballooning and NASH score (51). Particular factors in the diet may be important, with an emphasis on reducing sugar (especially fructose which has been implicated in pathogenesis) and saturated fat (52).

There is uncertainty about the optimal pharmacological treatment of paediatric NAFLD (53). Insulin sensitizers such as metformin are used frequently in improving insulin resistance and are a mainstay of treatment in type 2 diabetes. In the absence of diabetes, metformin has also been used in NAFLD. Recent randomised controlled trials in both adults and children, however, have failed to demonstrate any difference to placebo (54, 55). Thiazolidiones have been used in adults but not extensively evaluated in children in view of reported early safety concerns (56). The PIVENs trial in adults did not find that pioglitazone was superior to placebo for non diabetic patients with NAFLD (55).

Reducing oxidative stress is another therapeutic target. Most interest has been around role of vitamin E. In both the paediatric TONIC trial and the PIVENs adult trial vitamin E at large doses (800 IU/day in adults and 400 IU/day in children) was found to improve histological severity when compared to placebo (54, 55). Ursodeoxycholic acid has also been investigated but a recent Cochrane meta-analysis concluded that it did not have any significant therapeutic advantage (57). Other methods of weight loss such as bariatric surgery have been found to have inconclusive effects on NAFLD (58). With the growing need for bariatric surgery in certain morbidly obese children and adolescents, the long term outcomes need to be carefully monitored.

Polyunsaturated fatty acids (PUFA) have become a particular focus of interest of late with a number of small trials demonstrating promising effects in patients with NAFLD. PUFA such as docosohexaenoic acid (DHA) may increase beta oxidation and decrease lipogenesis, improving both steatosis and progression to steatohepatitis (59). A paediatric study found improvement in insulin resistance and steatosis on ultrasound, but not in serum ALT following 6 months of therapy with DHA (60). Probiotics are also a promising therapy in light of the evidence that the gut/liver axis and bacterial overgrowth have a role in the development of NAFLD. Several small studies including two in children have suggested their therapeutic effect (61, 62). The role of bariatric surgery in the condition remains investigational (63).

Future agenda

In view of the significant growth in prevalence of paediatric NAFLD, the disease has become a major cause for concern not only for paediatric hepatologists/gastroenterologists, but also for paediatricians in general, family doctors and public health specialists. NAFLD has become an epitome of general health and marker of future risks for not only liver disease but also endocrine, cardiovascular and skeletal pathology. A better understanding of the condition and improvement in management are urgently needed in order to avoid a potential epidemic of end-stage liver disease in early adulthood. Firstly the pathophysiology of the condition and the differences to the adult disease need to be further clarified. Prevention is undoubtedly the key in the management but implementation of the lifestyle changes requires combined efforts by policy makers and the society. Though lifestyle change is the mainstay management, achieving and maintaining such change is a major challenge. NAFLD is not just about diet and exercise. The treatment-resistant and advanced NAFLD in children is often associated with disturbed family dynamics and mental health

problems such as depression. The more susceptible individuals in the population need to be targeted for better prevention. The additive effect of other treatments undergoing trial at present may prove beneficial.

Conclusion

The prevalence of paediatric NAFLD is increasing at an alarming rate. The pathophysiological mechanisms are not yet fully clear but it is clear that many factors may contribute. The mainstay of management is dietary and lifestyle modification but there is some evidence for anti-oxidants, probiotics and polyunsaturated fats in treatment. Further research is needed in order to understand better the factors contributing to development and progression of the condition in addition to ways of preventing and reversing liver injury.

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

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