Pediatric Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) is the number one chronic liver disease worldwide and is estimated to affect nearly 40% of obese youth and up to 10% of the general pediatric population without any obvious signs or symptoms. Although the early stages of NAFLD are reversible with diet and lifestyle modifications, detecting such stages is hindered by a lack of non-invasive methods of risk assessment and diagnosis. This absence of non-invasive means of diagnosis is directly related to the scarcity of long-term prospective studies of pediatric NAFLD in children and adolescents. In the majority of pediatric NAFLD cases, the mechanisms driving the origin and rapid progression of NAFLD remain unknown. The progression from NAFLD to non-alcoholic steatohepatitis (NASH) in youth is associated with unique histological features and possible immune processes and metabolic pathways that may reflect different mechanisms compared with adults. Recent data suggest that circulating microRNAs (miRNAs) are important new biomarkers underlying pathways of liver injury. Several factors may contribute to pediatric NAFLD development, including high-sugar diets, in utero exposures via epigenetic alterations, changes in the neonatal microbiome, and altered immune system development and mitochondrial function.

pediatric NAFLD | clinical biomarkers | microRNAs | developmental programming | microbial dysbiosis | trained immunity

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a generic term that describes a spectrum of diseases including non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), fibrosis, and NAFLD-cirrhosis [1]. The global epidemic of NAFLD is increasing exponentially owing to the growing prevalence of obesity and type 2 diabetes (T2D) in children and adults along with the aging population [2]. Recent estimates indicate that the global prevalence of NAFLD is 25%, with the highest prevalence in the Middle East and South America and the lowest in Africa [3]. NAFLD is the most common liver disorder and currently is the second most common cause of liver transplantation [4]. NAFLD is estimated to affect 34% of obese children aged 2–19 years and 10% of the general pediatric population [5]. Pediatric NAFLD is associated with extrahepatic complications such as early atherosclerosis and cardiac dysfunction [6][7] and abnormal renal function [8]. Recently, NAFLD has been renamed by some as metabolic (dysfunction) associated fatty liver disease (MAFLD) as the majority of patients with fatty liver have metabolic dysfunction in the form of T2D, dyslipidemia, and increased insulin resistance [9]. Pediatric NAFLD has a complex pathophysiology and is different from adult NAFLD with multiple inputs, including perinatal events. Understanding these differences may lead to new biomarkers and opportunities for novel therapeutics.

2. Clinical Pathophysiology

Despite advances in understanding NAFLD in adults, major gaps remain in defining pathways and mechanisms unique to NAFLD pathology in children. The pathophysiology of pediatric NAFLD is multi-factorial and includes complex interactions among hormonal, nutritional, genetic, and environmental factors [10] that may begin in utero [11]. Initially, NAFLD involves hepatic steatosis, which comprises lipid accumulation arising from excessive influx of fatty acids from endogenous fat depots, excess consumption of dietary fat, and hepatic de novo lipogenesis (DNL). NASH is characterized by inflammation, oxidative stress, mitochondrial dysfunction, and fibrosis [12].

Guidelines for diagnosing NAFLD in children were updated in 2017 by the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) [13]. That expert group emphasized that obese children should be prioritized for screening because of their higher likelihood to have NAFLD. They also recognized that an unmet challenge is to identify reliable and minimally invasive biomarkers for the disease. The only currently NAPSGHAN-recommended blood test for screening for pediatric NAFLD is alanine aminotransferase (ALT). The American Academy of Pediatrics endorsed the NASPGHAN recommendation to measure ALT beginning at ages 9–11 years for all obese children, and for overweight children with additional risk factors such as insulin resistance, diabetes, dyslipidemia, sleep apnea, central adiposity, or a family history of NAFLD [14]. NAFLD is likely present in obese children who have ALT values that are 2-fold higher than the sex-specific normal range. An advantage of ALT is that it is inexpensive, and the measurement has been standardized among laboratories. However, normal ranges reported among locations are variable, which complicates interpretation, and, more importantly, ALT values do not reliably differentiate NAFLD severity, or distinguish uncomplicated NAFLD from NASH in children or adults [15][16]. Several other potential circulating biomarkers have been proposed [17][18][19][20][21][22][23][24] but few have been tested in children with biopsy-proven NAFLD, or tracked over the course of treatment or disease progression [25].

Imaging tools have been used for screening, but most approaches have recognized limitations [24]. Standard B-mode ultrasound lacks specificity and sensitivity for steatosis and is not recommended. Computed tomography and magnetic resonance (MR) modalities are better than ultrasound; however, they carry concerns about radiation (the former), expense and availability of instrumentation (the latter), and the need for sedation in some children (both) [13]. Newer imaging tools that are gaining acceptance and application include MR elastography, ultrasound-based shear wave elastography using acoustic radiation force impulse (ARFI) techniques, and ultrasound-based vibration-controlled transient elastography (FibroScan) [26][27]. MR elastography has not yet been shown to be as useful in children as it has in adults and requires an MRI machine and a specific surface coil, so it is likely to be implemented only in medical centers with specialty clinics. ARFI was shown to be useful in determining liver fibrosis in pediatric patients with chronic liver disease [28] and showed high correlation with aspartate aminotransferase (AST)/ALT ratios and detecting NAFLD in childhood obesity [29]. The FibroScan has been validated for measuring liver steatosis in adults and children and is FDA approved for clinical and research applications [30]. There are small, medium, and extra-large probes that can be selected to accommodate the size of the patient, which obviously spans a large potential range from younger children to adolescents and young adults.

Like MR-based approaches, it is not yet widely available in pediatric clinics [31]. Thus, imaging techniques have promise but additional tools are needed for comprehensive liver health profiling.

The current gold standard for confirmation of NAFLD is histological examination of liver tissue obtained by biopsy, as it rules out other causes of liver dysfunction. Histological differences have been demonstrated in pediatric versus adult NAFLD, with children being more likely to display portal inflammation and fibrosis and less ballooning and peri-sinusoidal fibrosis than adults. Whether those distinctions result in different trajectories of disease progression or responses to treatment is not yet known. It should be noted that, in a separate clinical guideline for pediatric NAFLD released by the American Association for the Study of Liver Diseases in 2018, there were no recommended blood or imaging tests for screening for NAFLD in obese children because of the paucity of evidence [32]. Recommendations for clinical work up and liver biopsy, however, were similar to those made by NASPGHAN .

Bile acids are commonly studied as biomarkers and therapeutic targets for NAFLD. Bile acids are synthesized from cholesterol in the liver and are the major components of bile. Altered bile acid composition and metabolism have been reported during the progression of NAFLD [33]. Currently, little evidence exists linking the development of cholestasis with NAFLD/NASH. Metabolomic analysis revealed significantly increased serum levels of glycochenodeoxycholate, glycocholate, and taurocholate in patients with NAFLD compared with healthy controls [34]. Research in this field is complicated by the complexity of the liver-bile-intestinal axis and is therefore more focused on pharmacotherapies like the FXR-agonist, obeticholic acid, and peroxisome proliferator-activated receptor (PPAR) agonists such as saroglitazar rather than on bile acids as clinical biomarkers. Free fatty acids and their metabolites, which contribute to liver injury via increased oxidative stress, are typically elevated in children and adults with obesity and NAFLD [35][36][37][38]. As with bile acids, free fatty acids are mostly used as therapeutic targets rather than clinical biomarkers.

Genetic factors are associated with NAFLD susceptibility and progression. A variant in the patatin-like phospholipase domain-containing protein 3 gene (*PNPLA3*) is associated with increased liver fat, fibrosis, and risk for carcinoma, with a higher prevalence of the at-risk allele in Hispanic youth [39][40]. A variant in the glucokinase regulatory protein (*GCKR*) gene was associated with an increased rate of DNL in obese adolescents [41], and a minor allele in the transmembrane 6 superfamily 2 human gene (*TM6SF2*) was associated with higher fibrosis and NAFLD Activity Score in children [42]. Lysosomal acid lipase (LAL) deficiency is observed in two recessive genetic disorders involving increased lysosomal cholesterol ester storage. LAL activity was shown to be significantly reduced in children [43] and adults [44][45] with NAFLD, suggesting a possible role of LAL reduction in the progression of NAFLD [46]. It is not yet known whether the presence of these polymorphisms modify the response to lifestyle or pharmacological interventions designed to slow or reverse the development and progression of NAFLD. However, Van Name et al. [47] demonstrated that a small group (n = 17) of obese children who completed a 12-week diet with low n-6/n-3 fatty acids had favorable changes in lipids, liver fat, and insulin sensitivity and these changes were the same or slightly better in patients with the *PNPLA3* “at risk” genotype.

3. Role of Nutrients in Pediatric NAFLD

Animal and human evidence supports the adverse effects of high sugar intake, particularly fructose, on obesity and pediatric NAFLD risk, including in utero exposure [48][49][50][51]. Fructose consumption acutely stimulates hepatic DNL in adolescents and adults [52][53]. In mice chronically consuming a high-fat diet (HFD), the addition of dietary sugars promoted triglyceride production [54][55]. Extra glucose promoted lipid synthesis through activation of the transcription factor carbohydrate-responsive element-binding protein (*Chrebp*), while extra fructose activated both *Chrebp* and sterol regulatory element-binding protein 1 (*Srebp1*) [54]. Dietary glucose and fructose exerted different effects on mitochondrial protein acetylation and malonyl CoA, resulting in a greater reduction in fatty acid oxidation and greater lipid synthesis in response to the extra fructose diet compared with the extra glucose diet [55].

It is perhaps not surprising that a reduction of dietary sugars results in several improvements in liver health. In one study, obese adolescents who were habitually high consumers of dietary sugar were placed on a prescribed diet for nine days that limited added sugar and fructose to 10% and 4% of energy intake, respectively [56][57]. In response to this short-term intervention, liver fat, DNL, and fasting insulin decreased, accompanied by a small (1.1 kg) amount of weight loss [56][57]. Subsequent interventions lasting eight weeks that were designed to be more sustainable in pediatric clinical settings yielded consistent results [58][59]. Schwimmer et al. [58] enrolled boys 11 to 16 years old with NAFLD and at least 10% hepatic fat content. They were randomly assigned to either a usual diet (control) or a low-sugar diet with a goal of less than 3% of daily energy from added sugars (intervention). For intervention participants, meals for their entire family were provided and existing foods with excess sugar were removed from their home. In response to the low-sugar diet, hepatic fat content was reduced by about 8% versus only 1% change in the control group. There was also greater reduction in plasma liver enzyme activities in the low-sugar group, but no significant changes were observed in fasting insulin or triglycerides. In a similar manner, Goss et al. [59] compared the effect of moderate reductions in dietary carbohydrate versus dietary fat in boys and girls with obesity and NAFLD. All meals were provided for the first two weeks and then families were given instructions on how to follow dietary guidelines for the remaining six weeks. Following the intervention, hepatic lipid was reduced in the low-carbohydrate group by 6% but only 1% in the low-fat group. The low-carbohydrate group also maintained fasting insulin sensitivity (HOMA-IR), whereas the low-fat group had an increase in HOMA-IR. Each of these studies demonstrate that dietary sugar reduction can lead to improvement in liver steatosis and potentially other health outcomes in obese adolescents.

Studies in humans have shown that individuals with NAFLD have low omega-3 polyunsaturated fatty acid (n-3 PUFA) intake and high n-6/n-3 PUFA intake ratio [60][61]. Owing to their effect on hepatic lipid metabolism and inflammation, n-3 PUFA as a nutrient supplementation has been recommended for improving NAFLD [62][63]. A randomized control study by Nobili et al. [64] reported that docosahexaenoic acid taken orally for 6 months reduced liver fat content and improved insulin sensitivity in children with NAFLD. Furthermore, Janczyk et al. [65] reported that although n-3 PUFA supplementation for 6 months did not improve steatosis as determined by ultrasound and ALT levels, it improved AST and gamma-glutamyl transpeptidase levels in children with NAFLD compared with placebo. Another study found that n-3 PUFA supplementation for 12 months had beneficial effects on steatosis and ALT levels in children with NAFLD and obesity [66]. Overall, nutrient intervention with modified PUFA levels appears to be safe and efficacious for the treatment of NAFLD in children. However, the real challenge for clinicians,

behaviorists, and public health officials is to transfer these effects from small, highly controlled feeding studies to strategies that can be broadly implemented, with affordable, palatable, and sustainable diet options for families.

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