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Nonalcoholic fatty liver disease in children and adolescents - Relationship with Polycystic Ovary Syndrome

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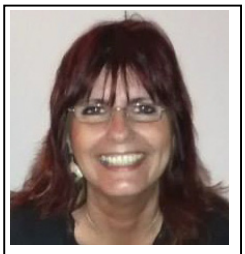

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is defined as the accumulation of triglycerides (TGs) within hepatocytes exceeding 5 % of liver weight. NAFLD is a spectrum of pathological processes from nonalcoholic fatty liver or simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma. As NAFLD induces metabolic syndrome (MS), then, NAFLD is associated with insulin resistance (IR), type 2 diabetes mellitus (T2DM), hypertension and even Polycystic Ovary Syndrome (PCOS). Because it is well established that patients carrying gene mutations also develop NAFLD in the absence of IR, the genetic predisposition to NAFLD is also discussed. Little is known about the diagnosis and treatment of NAFLD in children and adolescents and the lack of non-invasive diagnostic tools in these populations is a major problem faced by physicians. The present review aims to discuss recent findings of NAFLD in children and adolescents and, considering the features in common with PCOS, we also discuss their relationship.

Keywords: Nonalcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), triglycerides (TG), adolescents, children, metabolic syndrome (MS), insulin resistance (IR), obesity.

1. INTRODUCTION

The accumulation of triglycerides (TGs) in liver cells leads to fatty liver diseases. One of the most common of these liver disorders worldwide is nonalcoholic fatty liver disease (NAFLD) which occurs when the accumulation of TGs within hepatocytes exceeds 5% of liver weight. NAFLD comprises a spectrum of pathological processes ranging from nonalcoholic fatty liver or simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Hepatokines, released to the liver with steatosis and key mediators in the pathogenesis of local and systemic inflammation, impair peripheral insulin sensitivity [2]. The subsequent chronic inflammatory condition induces liver damage [3]. It was first considered that NAFLD was the hepatic manifestation of metabolic syndrome (MS) because of common manifestations such as insulin resistance (IR), obesity, type 2 diabetes mellitus (T2DM), hyperlipidemia, hypertension and Polycystic Ovary Syndrome (PCOS) [4]. However, several prospective studies have revealed that liver fat accumulation precedes IR and MS [5]. Thus, the role of NAFLD in the onset of MS during childhood is a very important event and a vicious circle involving liver steatosis and decreased insulin sensitivity has been described [6,7].

Regarding the molecular mechanism involved, it has been reported that free fatty acids (FFAs) and TGs act either directly or via toll-like receptors, developing endoplasmic reticulum stress, mitochondrial dysfunction, increased production of reactive oxygen species (ROS), impairment of liver protein metabolism, inhibition of insulin signaling and activation of several inflammatory pathways [8]. Liver biopsy remains the gold standard for definitive diagnosis of NAFLD. However, this method might either overestimate or under- estimate liver damage because it only samples a very small portion of the liver. In recent years, noninvasive test, particularly, aminotransferase levels and abdominal ultrasonography have been preferred [9, 10].

In children and adolescents, NAFLD is the most common form of chronic liver disease and can progress to liver cirrhosis [11-13]. The disease is caused by central obesity with IR and additional factors influencing inflammatory activity [13, 14].

In this review, we introduce the recent findings on NAFLD in children and adolescents and considering the features common with PCOS, we also discuss their relationship.

2. CAUSES OF NAFLD

It is difficult to define particular markers of NAFLD development. It has been recently reported that non-high-density lipoprotein cholesterol is an independent predictor for NAFLD even stronger than other lipoproteins [15]. On the other hand, a strong association between NAFLD and T2DM has been described since it has been found that T2DM is an independent predictor of the progression of hepatic fibrosis in patients with NAFLD [16]. Moreover, as the presence of T2DM increases the risks of cardiovascular disorders and the development of hepatocellular carcinoma [17], physicians in charge of NAFLD patients should detect T2DM in the early stage. In fact, the oral glucose tolerance test should be indicated in NAFLD subjects [18]. In addition, other diseases or factors such as abetalipoproteinemia, hypobetalipoproteinemia, familial combined hyperlipidemia, glycogen storage disease, Weber–Christian syndrome, lipodystrophy, total parenteral nutrition, hepatitis C infection, severe surgical weight loss, medications, starvation, Wilson´s disease, environmental toxicity, or celiac disease are infrequent and easily overlooked [19].

Particularly in children, several pathologies such as Turner syndrome, abnormal mitochondrial and fatty acid metabolism, nephrotic syndrome, and Down syndrome are related to fatty liver accumulation [20-24].

3. PREVALENCE OF NAFLD

NAFLD, which is identified on imaging studies in 20–33% of adults, is currently becoming one of the most prevalent liver dis-

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eases. In Europe and the USA, NASH is diagnosed in 3–16% of potential liver donors. It is also a frequent cause of cirrhosis and is projected to be the leading indication for liver transplantation in the US by 2020 [25, 26].

In children, NAFLD is the most common form of chronic liver disease with the aggravating that it may develop liver cirrhosis [11, 13]. The prevalence in children is almost 10% and NAFLD is conditioned by central obesity, IR and inflammatory factors leading to steatohepatitis [13, 14]. In a retrospective review of 742 children aged between 2 and 19 years, adjusted for age, gender, race, and ethnicity, Schwimmer *et al.* [13] reported that fatty liver is estimated to be 9.6% and that its prevalence increases with age, being 0.7% for 2-4 year old children and 17.3% for 15 to 19 year-old adolescents. These authors also found that race and ethnicity influence the prevalence of fatty liver, being 10.2% in Asian children, 1.5% in black children, 11.8% in Hispanic children and 8.6% in white children. They found that the highest rate of fatty liver (38%) was that in obese children. Other authors such as Park *et al.* (2005) [27] and Tominaga *et al.* [28] found similar percentages in the USA and Asian countries, and reported that the prevalence of NAFLD/NASH is as high as 2.6%-9.6%, taking in account differences in race and ethnicity.

The prevalence of IR in obese children increases the prevalence for T2DM. It is estimated that 170 million children under 18 years of age worldwide are overweight or obese, representing more than 20% of all children in many countries [29]. In a retrospective longitudinal hospital-based cohort study, Feldstein *et al.* [30] reported that 4 out of 66 children with NAFLD developed T2DM between four and eleven years after diagnosis and that two children died and two underwent liver transplantation for cirrhosis.

4. CLINICAL DIAGNOSIS

Although the pathogenesis of NAFLD remains unknown, Matteoni *et al.* [31] classified NAFLD into four types: type 1 a simple fatty liver, type 2, a type showing steatohepatitis (fatty liver and lobular inflammation), type 3, a type showing steatonecrosis and ballooning and swelling of hepatocytes, and type 4, a type showing steatonecrosis and Mallory bodies (liver cell ballooning degeneration) or fibrosis. These authors reported that types 1 and 2 do not progress to cirrhosis and types 3 and 4 are pathologically defined as NASH.

Unfortunately, there are no specific symptoms associated with NAFLD and NASH in children. However, some observations as obesity, sleep apnea, hypertension, hyperinsulinemia, and acanthosis nigricans can be useful [32]. The presence of visceral obesity, a body mass index greater than + 2SD, or an increase in weight of 10% or more per year should lead to suspect NAFLD [32]. In children, NAFLD diagnosis can be confirmed by no invasive tests, such as; the presence of hepatitis B surface antigens, hepatitis C virus antibodies, anti-mitochondrial antibodies, anti-nuclear antibodies, ceruloplasmin, α-antitrypsin, and transferrin [33]. In addition, the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are 2-4 times increased, and the level of ALT is higher than that of AST [34]. Therefore, the levels of ALT and AST are higher in NASH than in NAFLD and patients with cirrhosis show an ALT/AST ratio less than 1 [34]. Regarding noninvasive methods to diagnose NAFLD in children, imaging methods to measure liver fat have been recently developed [35].

Given the association of NAFLD with MS, markers associated with MS work as NAFLD predictors. For example, lipid accumulation product (LAP) is an index which combines waist circumference and TG levels reflecting lipid accumulation [36]. LAP is related to central obesity and IR [37], both predisposing factors of NAFLD. LAP has been shown to be positively correlated with the presence of hepatic steatosis [38, 39] as well as with increased values of ALT in adults [40].

Another index that has been proved to be a powerful tool in determining the probability of an individual having hepatic steatosis is the Fatty Liver Index (FLI) [41]. This index considers not only metabolic markers as body max index (BMI), waist circumference and TG serum levels in its formula but also the Gamma-glutamyltransferase (GGT) serum levels. Increased levels of GGT have been described in patients with NAFLD and have been associated with increased mortality [42, 43].

Both LAP and FLI have been questioned because of their effectiveness in predicting the severity of hepatic steatosis. Nevertheless, in clinics, their utility is focused on the possibility to determine the presence of steatosis, which is sufficient to evaluate the treatment for the healthcare of the patient [38]. In children and adolescents, these non-invasive markers may be of great interest because of their association with other metabolic derangements such as PCOS and MS. Furthermore, studies in premenopausal women with PCOS have found that LAP and FLI in these patients are increased as compared to control groups, showing that PCOS patients are in risk of presenting and developing NAFLD and that this could worse as age increases [44-46]. On the other hand, the characteristics of NAFLD/NASH in adolescents and adults are different from those in children. In children, the ratio is characterized by fatty changes, inflammation and fibrosis of the portal area, and absence of perisinusoidal fibrosis and hepatocyte ballooning [47]. Generally when patients show strong fibrosis, they are classified as having type 2 NAFLD/NASH. Thus, in contrast to adolescent or adult NAFLD, pediatric NAFLD is classified into two types [48]. However, in children, the grading of necrosis and inflammation is very low [32].

5. GENETIC AND EPIGENETIC BASES OF NAFLD

Although the main evidences consider the link between NAFLD and IR, genetic predisposition and environmental factors, including the diet, have also been reported [29].

A recent meta-analysis carried out by Zain *et al.* [49] has provided evidence of significant association between a common variant in the glucokinase regulatory (GCKR) rs780094 gene and risk of NAFLD. These authors found that the GCKR rs780094 gene shows a similar effect size in both Asian and non-Asian populations. On the other hand, Del Ben *et al.* [50] reported a low prevalence of MS and reduced cardiovascular risk in NAFLD patients with the patatin-like phospholipase domain-containing protein3 (PNPLA3MM) genotype. These data and those reported by Marzuillo *et al.* [51], where the polymorphisms of PNPLA3I148M and TM6SF2 are associated with the development of NAFLD, represent new issues to the understanding of NAFLD pathogenesis. On the other hand, as the presence of the PNPLA3I148M variant displays increased risk of NAFLD with reduced levels of central adiposity, body mass index, TGs and IR, these data suggest differential roles in fat storage and distribution according to the metabolic status [52]. Taken together, these findings allow suggesting a possible role of PNPLA3 and TM6SF2 in extrahepatic conditions such as in PCOS, an issue that deserves further studies. In addition, genomic studies carried out by Li *et al.* [53] have shown that the microsomal transfer protein (MTP) -493G/ T polymorphism may contribute to the development of NAFLD.

Using liver mRNA from NAFLD patients, Arata *et al.* [32] carried out a genome-wide association study (GWAS) and showed that a combination of increased expression of lymphocyte cytosolic protein-1 (LCP1) and decreased expression of group-specific component (GC) is associated with susceptibility to NAFLD/NASH. On the other hand, Adams *et al.* [54] showed that GC gene polymorphisms and LCP1 levels correlate with vitamin D levels and hyperlipidemia, respectively.

Beyond the genetic basis, which generates a predisposition to develop NAFLD, the environmental factor also plays an important role in the pathogenesis of these liver diseases. Several studies have proposed that the maternal environment could affect the develop-

ment of the fetuses generating metabolic derangements in postnatal life through a fetal programming [55, 56]. This hypothesis has been proposed as a redefinition of the "first hit" in the development of NAFLD [57]. Fetal programming occurs via epigenetic changes that occur as an adaptation to the maternal environment. When this environment is different from the one found in postnatal life, there is a no adaptation to the new environment and this could lead to the development of metabolic diseases. Epigenetic changes affect transcription rates and lead, in postnatal life, to a differential expression of markers that may be involved in the pathogenesis of NAFLD and NASH, such as the ones that regulate glucose and insulin homeostasis and lipid metabolism. Among these markers, peroxisome proliferator-activated receptors (PPARs) have been implicated in NASH pathogenesis. Human liver PPARα gene expression is negatively correlated with NASH severity, visceral adiposity and IR and positively correlated with adiponectin. Histological improvement is associated with an increase in the expression of PPARα and its target genes. These data suggest that PPARα is a potential therapeutic target in NASH [58]. In experimental models of NAFLD, Sookoian and Pirola [59] found that the expression of PPARGC-1A, a master regulator of mitochondrial biogenesis and a co-activator of PPARγ and PPARα (regulators of lipid storage and mobilization) is reduced and also found evidences of hypermethylation of its promoter.

In addition, to the chromatin changes involved in the pathogenesis of NAFLD, it has also been found that non-coding mRNA is involved in its development and in the progression to NASH and to even more severe liver damage and that it may also be important in its diagnosis [60,61]. Some studies have shown that the miRNAs assigned to NAFLD/NASH risk have also been described as associated with metabolic and inflammatory pathways [62,63].

6. RELATIONSHIP BETWEEN NAFLD AND PCOS

PCOS is a common endocrine disorder in females of reproductive age. PCOS is characterized by menstrual irregularities (oligomenorrhea or amenorrhea) and clinical and/or biochemical hyperandrogenism in the presence or absence of polycystic ovaries with more than half of the patients being overweight or obese [64]. It has been shown that approximately 37-47% of adolescents with PCOS have MS compared with 0.6-8.9% of healthy adolescents [12]. Lewy *et al.* [65] found that 50% of adolescents with PCOS, matched for age, body composition and Tanner stage, show MS and IR, whereas Palmert *et al.* [66] reported that 30% of adolescents with PCOS, including lean PCOS, have impaired glucose tolerance. Since PCOS and NAFLD share common anomalies regarding their pathogenesis, it is not uncommon that liver fat is associated with increased age, increasing abdominal adiposity, worsening insulin sensitivity and dyslipoproteinemia in adolescent girls with PCOS [44]. A recent meta-analysis has shown that women with PCOS display higher risk of NAFLD than healthy controls [67].

Both NAFLD and PCOS present similarities not only in their clinical features regarding glucose homeostasis, lipid alterations and their relation with MS, but also in their pathogenesis. The etiology of NAFLD and PCOS is under discussion, and both diseases are understood as having a multifactorial origin, with an important maternal factor. PCOS etiology is also related to a genetic basis with genes involved in steroidogenesis as well as others associated with obesity and insulin resistance [68, 69]. A functional mutation in PPARγ has been found to be associated with the syndrome [70] and has also been related to the risk of NAFLD in Asian and American people [71-74]. Besides, fetal programming mediated by androgens has been postulated as involved in PCOS etiology [75]. It has been shown in animal models that an androgen excess during pregnancy programs not only PCOS in puberty but also metabolic derangements and tends to lipid accumulation [76,77] (Fig. **1**).

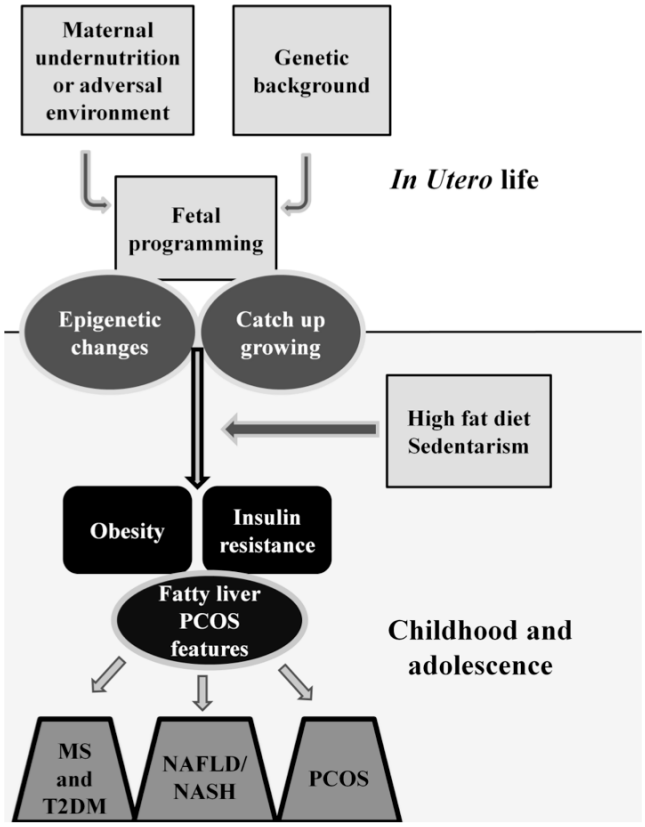

Fig. (1). Factors contributing to the development of NAFLD, PCOS and metabolic-associated disorders.

In summary, screening patients with PCOS for NAFLD is an issue of debate. However, even when no reports have revised the relationship between NAFLD in PCOS patients, the population of children and adolescents displaying obesity, MS, and hyperandrogenism, ovarian cysts and anovulatory estrous cycles should be controlled for possible liver injury.

7. MANAGEMENT OF NAFLD IN CHILDREN AND ADOLESCENTS

NAFLD is often associated with obesity, diabetes, hyperlipidemia, and hypertension and is considered to be a type of MS. A progressive increase in intrahepatic TG levels is associated with impairment of insulin action in the liver [78]. Thus, pediatric and adolescent NAFLD treatment focuses on reducing hepatic injury and future cardiovascular risk and related mortality and components of MS [79] (Table **I**).

7.1. Lifestyle Modifications

The first line of treatment of NAFLD in children and adolescents is improvements in lifestyle including diet and exercise; however, it is important to point out that quick weight loss is associated with increased liver fibrosis [32].

Prospective studies in children with NAFLD have shown significant improvements in the metabolic profile and histological abnormalities of NAFLD when lifestyle intervention comprises diet and increased physical activity [80, 81]. Although no clinical recommendations exist to define lifestyle modification or characterize the type of diet needed in children with NAFLD, some tips have been defined [79]. Among them, it has been recommended to adopt

Table I. Efficacy of main treatments against nonalcoholic fatty liver disease symptoms.

Treatment	Efficacy
Lifestyle modifications There is no a specific diet but some tips have been defined. It is necessary an adequate supply of omega-3 fatty acids, to include fruits, whole-grain food. It is essential daily physical activity of 30-60 min of moderate-to-vigorous physical activity.	Improves metabolic profile and histological abnormalities of NAFLD.
Insulin sensitizers Metformin	Controversial (effective but no more than improvement of lifestyle).
Antioxidants Vitamin E Vitamin C	Significant improvements in NAFLD and NASH activity scores. No changes in ALT levels or liver inflammation. Fibrosis is controlled.

NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; ALT: Alanine aminotransferase.

an age-appropriate low-fat dietary strategy and avoid simple refined carbohydrates [82, 83]. In addition, it has been recommended: to follow a regular meal and snack schedule, to use portion-controlled servings of nutritionally high-quality food, and to reduce sugar intake and avoid artificially sweetened fruit juices and beverages [82, 83]. In addition it is important to ensure an adequate supply of omega-3 fatty acids by consuming two to three portions of fish per week, to substitute refined grains for whole-grain food and finally to reduce screen time (video games/television watching) to no more than 1–2 h per day and encourage daily physical activity that includes 30–60 min of moderate-to-vigorous physical play or activity [82, 83].

7.2. Drug Therapies

Although drug therapies for both children and adolescents with NAFLD focus on insulin sensitizers and antioxidants, the efficacy of both treatments has not been completely established to date.

7.3. Insulin Sensitizers

Given the close relationship between NAFLD and IR, insulin-sensitizing drugs have been extensively evaluated in children and adolescents. Metformin is currently the only insulin sensitizer well studied in children and adolescents with NAFLD [48, 84, 85]. Schwimmer *et al.* [48] reported that metformin reduces ALT levels and hepatic steatosis; however, their study was limited by the small sample size (10 obese non-diabetic children with NAFLD) and the short duration of the therapy (24 weeks). In fact, Nobili *et al.* [84] found no differences between metformin therapy and lifestyle interventions when metformin therapy was extended for 24 months. Moreover, a large multicenter study carried out by Lavine *et al.* [85] involving 173 patients with NAFLD aged between 8 to 17 years has shown that 96 weeks of metformin treatment fails to demonstrate to be better than lifestyle intervention in decreasing ALT and AST levels.

7.4. Antioxidants

Oxidative stress is a major factor in the progression of NAFLD to NASH, as demonstrated by the increase of free radicals in NASH [86] and the lack of antioxidant defenses [87]. Thus, antioxidants are the second most extensively investigated agents for the treatment of NAFLD after metformin in children and adolescents [85, 88-90]. It has been reported that a low dietary intake of vitamin E is correlated with steatosis severity and that a low dietary intake of vitamin C is correlated with histological severity of NASH, fibrosis and inflammation [91]. Lavine [92] carried out the first clinical trial testing the effects of vitamin E in children with NAFLD and reported the normalization of the serum levels of AST and ALT. However, the small size of sample of this study compared with subsequent studies failed to demonstrate a superior effect of vitamin E as compared with lifestyle interventions [88]. In fact, Lavine *et al.* [85] later demonstrated that, compared with the placebo, the vitamin E arm showed significant overall improvement in NASH patients, attributable to improvement in hepatocellular ballooning ($P = 0.006$), but no changes in other components, including the degree of steatosis, inflammation or fibrosis.

7.5. Bariatric Surgery

As already mentioned, morbid obesity is strongly associated with NAFLD and the present best treatment for NAFLD and NASH is weight reduction through lifestyle modification. Because of frustrating inefficiency of such a therapeutic approach, bariatric surgery is increasingly performed in adolescents as an alternative option for weight reduction. Data on bariatric surgery in children with NAFLD are scarce, with a specific absence of clinical criteria, such as degree of obesity, a BMI value or a histological score that defines when surgery should be considered [93]. In a recent International Consensus, Nobili *et al.* [94] explore the indications and limitations of bariatric surgery in children with severe obesity with and without NASH. These authors also provide guidance for the exceptional indications for adolescents with extreme obesity with major comorbidity that may benefit from these controversial interventions. They reported that bariatric surgery can decrease the grade of steatosis, hepatic inflammation, and fibrosis in NASH but that uncomplicated NAFLD is not an indication for bariatric surgery. The authors found that the Roux-en-Y gastric bypass is considered a safe and effective option for adolescents with extreme obesity whereas laparoscopic adjustable gastric banding has not been approved by the Food and Drug Administration for use in adolescents. Finally, sleeve gastrectomy and other types of weight loss surgery that have grown increasingly common in adults still need to be considered investigational [94]. Thus, further studies, including a long-term risk analysis of patients who undergo surgery, are much needed to clarify the exact indications for bariatric surgery in adolescents.

CONCLUSION

NAFLD is becoming the main cause of liver disease in children and adolescents. The main challenge faced by physicians is the development of non-invasive diagnostic tools. Current treatments are focused mainly on lifestyle changes. However, no specific diets are recommended to optimize dietary strategies in children and adolescents with NAFLD. Until now, safe and efficient therapeutic strategies have not been established and large-scale randomized controlled trials in the pediatric and adolescent population should be developed. As PCOS and NAFLD share common features in their clinical metabolic manifestations and obese patients of PCOS are at high risk of metabolic derangements it is important to evaluate and control these patients since childhood, to detect whether they develop fatty liver as an another symptom of metabolic and insulin pathway derangements.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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