

**EPIDEMIOLOGY AND NATURAL HISTORY OF NAFLD**

## EPIDEMIOLOGIJA I PRIRODNA ISTORIJA NEALKOHOLNE MASNE JETRE

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**Summary:** Paralleling the growing prevalence of obesity and metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) is emerging as the most frequent hepatopathy in adults and children. The true prevalence of pediatric NAFLD is still unknown, because of the heterogeneity of diagnostic methods used for diagnosis in the available studies and the different characteristics of the populations evaluated. Pediatric NAFLD is typically of primary origin and it is strongly associated with several features of the metabolic syndrome. Age, gender and race/ethnicity are significant determinants of risk, and sex hormones, insulin sensitivity and adipocytokines are implicated in the pathogenesis of pediatric NAFLD. The natural history of NAFLD in children is still poorly understood, because of its complex nature and the scarcity of prospective studies, especially in pediatric populations. Both genetic and environmental factors seem to be implicated in the development and progression of the disease via multiple mechanisms that involve liver crosstalk with other organs and tissues, especially gut and adipose tissue. To evaluate and effectively treat pediatric NAFLD, the pathophysiology and natural history of the disease should be clarified and noninvasive methods for screening, diagnosis, and longitudinal assessment developed.

**Keywords:** nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, children, obesity, metabolic syndrome

**Kratak sadržaj:** Paralelno sa porastom prevalencije gojaznosti i metaboličkog sindroma, nealkoholna masna bolest jetre (NMBJ) sve se više prepoznaje kao najčešća hepatopatija kod odraslih i dece. Tačna prevalenca pedijatrijske NMBJ još je nepoznata, usled heterogenosti dijagnostičkih metoda korišćenih za postavljanje dijagnoze u dostupnim studijama i različitim karakteristikama ispitivanih populacija. Pedijatrijska NMBJ obično je primarno oboljenje i blisko je povezana s nekoliko odlika metaboličkog sindroma. Starost, pol i rasa/etnička pripadnost spadaju u značajne determinante rizika, dok u patogenezi pedijatrijske NMBJ učestvuju polni hormoni, osetljivost na insulin i adipocitokini. Prirodna istorija NMBJ kod dece još nije do kraja proučena, zbog njene složene prirode i nedostatka prospektivnih studija, naročito u pedijatrijskim populacijama. Po svoj prilici, u razvoju i progresiji ove bolesti učestvuju kako genetički tako i faktori sredine preko višestrukih mehanizama koji uključuju unakrsna dejstva između jetre i ostalih organa i tkiva, pre svega crevnog i adipoznog tkiva. Kako bi se procenila i efikasno lečila pedijatrijska NMBJ, trebalo bi razjasniti patofiziologiju i prirodnu istoriju ove bolesti i razviti neinvazivne metode za skrining, dijagnostikovanje i longitudinalne procene.

**Ključne reči:** nealkoholna masna bolest jetre, nealkoholni steatohepatitis, deca, gojaznost, metabolički sindrom

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## Introduction

Nonalcoholic fatty liver disease (NAFLD) has now become the most common cause of chronic liver disease in children. The term NAFLD is used to describe different degrees of fatty liver disease ranging from simple fat accumulation in the hepatocytes (>5%) to advanced forms of steatohepatitis (NASH) with necroinflammation and fibrosis (1). Although simple fatty liver and NASH are generally considered as the extreme points of a single disease spectrum, the most recent evidence seems to be changing this concept, considering fatty liver and NASH as two separate entities. Simple steatosis usually has a benign course, whereas NASH may progress to advanced forms of liver injury and may be associated with an increased risk of morbidity and mortality (2).

Initially, NAFLD was considered the result of a two hit process, but now multiple factors involved in the development and progression of disease have been identified and therefore it is believed that NAFLD has a multi-hits pathogenetic mechanism. The fat accumulation in the liver renders hepatocytes more susceptible to pathogenetic factors, such as oxidative stress, mitochondrial dysfunction, overproduction and release of proinflammatory cytokines, and endotoxin-mediated activation of the innate immune response, which induce persistent liver injury that leads to NASH.

The diagnosis of NAFLD is suspected on the basis of anthropometrical parameters (body mass index, waist circumference), laboratory assays (transaminases, lipid and glyco-insulinemic profiles), and/or ultrasound liver brightness. Although some progress has been made in the field of noninvasive

diagnostic tools for NAFLD, liver biopsy remains the imperfect gold standard to differentiate simple steatosis from NASH and to perform staging and grading of the disease in children (3, 4).

Currently, there are no specific therapeutic indications for the treatment of pediatric NAFLD. Lifestyle modification, including diet and regular physical exercise, represents the mainstay of treatment, but it is difficult to achieve (5, 6). Therefore, new treatments based on the pathogenetic mechanisms leading to NAFLD are under evaluation to establish the effective pharmacological therapy of this disorder (insulin-sensitizers, antioxidants, cytoprotective agents, and probiotics) (7).

## Epidemiology and risk factors

Although the number of reports on pediatric fatty liver in children has increased in the last two decades, the true epidemiological data on pediatric NAFLD are still unknown. There are, in fact, some difficulties in defining the population prevalence of pediatric fatty liver disease. The first is represented by the diagnostic test used in making the diagnosis of NAFLD (4). Although liver histology is recognized as the gold standard for diagnosing NAFLD (3), it obviously cannot be used in the general population for ethical and logistic reasons. Therefore, most of the available studies have been limited by the use of indirect markers of fatty liver, such as serum aminotransferases levels and/or evidence of bright liver at abdominal ultrasound (Table I). Several studies conducted in children and adults have demonstrated that many patients with NAFLD have normal aminotrans-

**Table I** Estimated prevalence of non-alcoholic fatty liver disease in children.

First Author	Country	Population	Diagnostic tool	Prevalence	Reference number
Tominaga	Japan	4–12 years – population study	US	2.6% overall	17
Schwimmer	USA	2–19 years – population study	Autopsy	9.6% overall, 38% obese	19
Franzese	Italy	Obese children	US ± ALT	53% with US, 25% with ALT	10
Chan	China	Obese children	US	77%	18
Park	Korea	10–19 years – population study	ALT	3.2%	16
Nobili	Italy	2–17 years – population study	ALT	20% overall, 50% obese	21
Strauss	USA	12–18 years – population study	ALT	3% overall, 6% overweight, 10% obese	15
Schwimmer	USA	Obese children	ALT	23%	20

Legend. US: abdominal ultrasound, ALT: alanine aminotransferase.

ferases levels, showing that liver enzymes are not satisfactory surrogate markers of NAFLD (8). Population cohort studies have reported that ALT levels are within the normal range in nearly 80% of patients with fatty liver (4, 9). Furthermore, some case series have not reported significant differences in the liver histology of patients with NAFLD among patients with abnormal and normal ALT values. Moreover, a discrepancy between ultrasonographic evidence of liver steatosis and aminotransferases levels has been reported in a group of Italian obese children by Franzese et al. (10). Of the 72 obese patients evaluated, 38 (56%) had liver steatosis at ultrasound examination, and only 18 (25%) had raised transaminases levels (10).

Ultrasound abdominal examination is the technique more frequently used to detect fatty infiltration of the liver, but it still has significant limitations. The principal is represented by the inability to distinguish between simple fatty liver and steatohepatitis, failing to detect liver fibrosis. Moreover, a hepatocytes fat infiltration  $\geq 15$ –30% is necessary to observe ultrasonographic modification (bright liver); in fact, its sensitivity decreases sharply if the degree of steatosis is less than 30% on biopsy (11). Furthermore, in addition to inter-observer variability (12), the ability of ultrasound to diagnose hepatic steatosis is highly reduced in the presence of severe obesity (13).

In cohorts of children of various nationalities selected for overweight or obesity, the prevalence of elevated ALT is higher and ranges from 8 to 42%, whereas the prevalence of bright liver ranges from 1.7 to 77%.

Pediatric NAFLD is widely distributed worldwide and variable in prevalence (3–10% in all individuals from South and North America, Europe, Asia and Australia) (14). However, this wide variability not only depends on the type of diagnostic approach, but is also influenced by age, sex, race and ethnicity of the sampled population, as well as by differences in metabolic risk factors.

Data from the National Health and Nutrition Examination Survey – NHANES III – ( $n = 2450$  children, age range 12–18 years) indicated that the prevalence of elevated ALT levels ( $> 30$  IU/L) was 7.4% among white adolescents, 11.5% among Mexican American adolescents and 6.0% among black adolescents (15). Data from South Korea and Japan are similar (16, 17). A population-based study using ultrasound documented the prevalence of 2.6% in Japanese children aged 4–12 years; this value increased to 22% in obese subjects (18). The SCALE (Study of Child and Adolescent Liver Epidemiology), a 10-year retrospective review of liver histology at autopsy of children aged 2–19 years, estimated that fatty liver occurs in 9.6% of subjects (19). Fatty liver prevalence increased with age, ranging from 0.7% for ages 2–4 years up to 17.3% for ages 15–19 years.

The prevalence of pediatric NAFLD peaks at puberty; factors that can explain the higher rate of NAFLD in adolescents include sex hormones and insulin resistance in puberty, or their increased control over unhealthy food choices and sedentary lifestyle.

Clinical series of children with NAFLD uniformly demonstrate the predominance of boys versus girls, with a male to female ratio of 2:1 (20). In addition to gender, race and ethnicity also play an important role in the development of NAFLD. Fatty liver is more prevalent in children and adolescents of Hispanic ethnicity and the least prevalent among black children and adolescents (20). Intermediate prevalence of fatty liver was noted in Asian and white children. Racial/ethnic differences may be related to genetic, environmental, or socio-cultural factors as well as differences in body composition, insulin sensitivity, and the adipocytokine profile (20).

Despite the diversity of diagnostic criteria used in population-based studies, obesity is the main risk factor for pediatric NAFLD (21). In fact, the prevalence of NAFLD in obese children increases up to 80% in several obesogenic countries, including USA, Europe and Japan (17, 18). Recently, Huang et al. (22) reported that in 219 schoolchildren, aged 6 to 12 years, the rates of NAFLD were 3% in the normal-weight range, 25% in the overweight range, and 76% in obese children. As reported in adults, NAFLD in children represents a metabolic condition which is strongly associated with other metabolic impairments (increased waist circumference, hypertension and insulin resistance) increasing the risk of developing type 2 diabetes mellitus, metabolic syndrome and cardiovascular disease. Recent studies agree in considering NAFLD as the hepatic feature of the metabolic syndrome. In view of this epidemic phenomenon, it is clear that pediatric NAFLD is now particularly worrisome, since the metabolic consequences in adulthood will significantly burden the health-care systems worldwide.

Although pediatric NAFLD is generally related to sedentary lifestyle and hypercaloric diet leading to progressive increases in BMI and visceral adiposity, recent studies suggested a strong heritability for NAFLD. Family clustering of NAFLD has been observed and some genetic determinants of NAFLD have been identified using genome-wide association studies. Single nucleotide polymorphisms (SNPs) in the genes involved in lipid metabolism (Lipin 1 – LPIN1, patatin-like phospholipase domain containing-3 – PNPLA3), oxidative stress (superoxide dismutase 2 – SOD2), insulin signalling (insulin receptor substrate-1 – IRS-1) and fibrogenesis (Kruppel-like factor 6 – KLF6) have been associated with the severity of liver damage in NAFLD patients (23). Moreover, an interesting interaction has recently been reported between genetic risk factors (PNPLA3 I148M) and dietary components, and the severity of steatosis (24).

Several ongoing studies on the genetic predisposition to NAFLD, undertaken by different research groups, will certainly and soon help in the identification of new diagnostic tools and molecular targets for the prevention and pharmacological treatment of NAFLD.

### Natural History

The natural history of pediatric NAFLD is still a mystery, because of its complex nature and the scarcity of prospective studies. Simple fatty liver, without histological evidence of inflammatory infiltrate or fibrosis, is generally a benign condition, whereas NASH may progress to advanced forms of liver damage, up to cirrhosis. Fatty liver progresses to NASH slowly, over many years or decades, but the prognosis may depend upon the histological subtype at the time of presentation. Several studies suggest that the risk of progression to advanced forms is related to histological severity (25). The presence of fibrosis characterizes the forms at risk of disease progression; once significant fibrosis is present, it is still unclear if it may reverse, and this is a topic of intense study and debate. An increased risk of fibrosis seems to be associated with insulin-resistance, dyslipidemia and visceral obesity, the principal features of metabolic syndrome (26, 27). The presence of obesity, particularly visceral obesity, is associated with a major risk of liver fibrosis in children. The elevated plasma free fatty acids (FFAs) levels, characteristic of obesity and linked to lipolytic activity of the adipocytes, cause mitochondrial damage promoting apoptosis and inflammation (28, 29).

Evidence that only a minority of patients with NAFLD progress to NASH suggests that disease progression is likely to depend on the complex interplay between environmental factors and genetic predisposition.

Recently, it has been reported by Feldstein et al. (2) that children with NAFLD have significantly shorter survival compared to the general population of the same age and sex, and that in some cases NAFLD in children may progress to cirrhosis and end-stage liver disease. In this study, in fact, during a follow-up period of twenty years, 2 of the 66 observed children underwent liver transplantation for decompensated cirrhosis and 4 of 13 histologically reevaluated patients showed progression of fibrosis (2). Moreover, some cases of cirrhosis secondary to NASH have been reported in pediatric settings, also in children aged ten (30).

Considering the number of patients affected in pediatric settings, it is imperative to identify in the large group of children with NAFLD those with advanced fibrosis, as well as the ones most likely to progress to end-stage liver disease. Therefore, it is important to develop noninvasive tests able to discriminate disease severity, identifying patients who require further investigation and closer follow-up.

### Conclusion

Despite the fact that during the past decade our understanding of pediatric NAFLD in terms of epidemiology, risk factors and pathogenesis has improved considerably, it is still unknown why some patients develop necroinflammation and/or fibrosis and others do not, and how the disease will evolve in adulthood. Its alarming worldwide diffusion requires growing awareness of the disease by general practitioners and pediatricians; moreover, further investigations are required to unravel its pathophysiology and identify novel therapeutic targets.

### Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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