



# Non Invasive Biomarkers for the Diagnosis of Non Alcoholic Steatohepatitis in Pediatrics



Rakesh Sharma<sup>1\*</sup>, Shimla<sup>2</sup> and Pancham Kumar<sup>2</sup>

<sup>1</sup>Professor Pediatrics, Indira Gandhi Medical College, India

<sup>2</sup>Assistant, Professor Pediatrics, Indira Gandhi Medical College,

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\*Corresponding author: Rakesh Sharma, Professor Pediatrics, Indira Gandhi Medical College, Shimla, India, Email: [drakeshigmc@gmail.com](mailto:drakeshigmc@gmail.com)

## Introduction

Nonalcoholic fatty liver disease (NAFLD) has emerged as a leading cause of chronic liver disease in children and adolescents in the developed and the developing world. A two- to three-fold rise in the rates of obesity and overweight in children over the last 2 decades is probably responsible for the epidemic of NAFLD. Emerging data suggest that children with NASH progress to cirrhosis which may ultimately increase liver-related mortality. More worrisome is the recognition that cardiovascular risk and morbidity in children and adolescents is associated with fatty liver. Pediatric fatty liver disease often displays a histologic pattern distinct from that found in adults. Liver biopsy remains the gold standard for diagnosis of NASH. Non-invasive biomarkers are needed to identify individuals with progressive liver injury. Targeted therapies to improve liver histology and metabolic abnormalities associated with fatty liver are needed [1].

## Review of Literature

NAFLD is a clinico-pathologic entity defined as presence of hepatic steatosis in individuals who drink little or no alcohol and represents a spectrum of liver disease ranging from bland steatosis to nonalcoholic steatohepatitis (NASH), which is a progressive form of liver disease that may lead to advanced fibrosis, cirrhosis and hepatocellular carcinoma in a subset of affected individuals. NASH is characterized by macrovesicular steatosis (the fat globules vary in size from very small to nearly filling the hepatocyte), ballooning degeneration with or without Mallory bodies, with lobular or portal inflammation, with or without fibrosis [2].

## Prevalence in India

The exact prevalence of NASH/NAFLD is not well established. Pooling data from various studies performed in tertiary medical centres of India the prevalence of NAFLD in obese children ranges from between 20%-77%.

**Age:** Several studies demonstrate that prevalence of fatty liver is higher in adolescents than younger children. Factors potentially explaining the higher rate of NAFLD in adolescents include hormonal changes surrounding puberty or their increased control over unhealthy food choices and sedentary physical activity with age. Hormonal changes during puberty may potentiate accumulation of fat in the liver [3].

**Sex:** NAFLD is more common in boys than girls [4]. These sex differences implicate estrogens as potentially protective, or indicate that androgens may aggravate NASH. Estrogen has been shown to be anti-apoptotic and anti-fibrogenic in in vitro studies in various cancer cell lines [5].

**Ethnicity:** Ethnic disparity in the prevalence of NAFLD has also been consistently seen across studies derived from multi-ethnic patient populations. NAFLD is more common in Mexican Americans than Caucasian Americans [5]. It is more common in young adults from Asian-Indian and Asian-American descent possibly due to higher rates of insulin resistance and visceral adiposity at equivalent BMI [6]. Pediatric NASH can be a challenging diagnosis because ballooning degeneration, classic zone-3 fibrosis and parenchymal inflammation that are commonly seen in adult NASH are less common in children with definite NASH [1].

## Histological Scoring System

Several histologic scoring systems have been proposed in patients with NASH which includes NAFLD scoring system designed and validated by the pathology committee of the NASH-Clinical Research Network (NASH-CRN) [7]. This system addresses the entire spectrum of lesions of NAFLD in both adult and pediatric populations. The scoring system is composed of 14 histologic features; four of the key features are evaluated semi-quantitatively including steatosis (0-3), lobular inflammation (0-2), hepatocellular ballooning (0-2), and fibrosis (0-4). The other

nine features, which include microvesicular steatosis, acidophil bodies, microgranulomas, lipogranulomas, portal inflammation, pigmented macrophages, megamitochondria, Mallory hyaline, and glycogenated nuclei, are qualitatively assessed as present or absent.

### **Development of novel serum biomarkers to diagnose NASH is essential for population-based screening. Biomarkers may be divided into four categories [1]:**

**Markers of Inflammation:** Cytokine imbalance has been demonstrated in both adults and children with NASH. Patients with NASH have higher serum IL-6 and TNF- $\alpha$  compared to either patients with steatosis alone or controls without liver disease [8,9-14]. Serum adiponectin levels are lower in patients with NASH compared to age/sex/BMI-matched controls but no set point accurately predicts NASH versus steatosis alone with reasonable sensitivity and specificity. The authors proposed a TNF- $\alpha$  mRNA cutoff value of 100ng/mL predicted NASH [area under receiver operating characteristic curves (AUROC) 0.685, sensitivity 66.7%, specificity 74.1%] [15]. The role of TNF- $\alpha$  in NASH is further supported by the beneficial effects of pentoxifylline, an antagonist of TNF- $\alpha$ , on biochemical and histological activity associated with NASH [16-18].

**Markers of oxidative stress:** Oxidative stress related to excess free fatty acids is considered key in the progression of steatosis to steatohepatitis in humans and animal models of NASH. Chalasani and colleagues showed that oxidized low-density-cholesterol (ox-LDL) and thiobarbituric acid-reacting substance (TBARS) in serum were higher in biopsy-proven NASH patients compared to age, gender, BMI-matched controls without liver disease in unadjusted analyses [10].

**Biomarkers of apoptosis:** Several groups report serum markers of hepatocyte apoptosis can discriminate NASH from benign steatosis. A major intermediate filament protein in the liver called CK-18 has been investigated in children with NASH [11]. Caspase-generated CK-18 fragments are higher in the liver and in serum in those with NAFLD compared to age- and sex-matched controls [12]. Serum CK-18 levels correlate with liver expression of CK-18 and both serum and liver CK-18 are independent predictors of NASH. A cut off value of 380 U/l provided a specificity of 94% and sensitivity of 91% for the diagnosis of NASH as compared to simple steatosis. The area under the curve was estimated to be 0.93 (95% CI: 0.83-1.00). Further studies are needed to confirm and validate these findings.

**Biomarkers of fibrosis:** Adult patients with NAFLD show that a combination of clinical, biochemical and/or extracellular matrix protein-associated serum markers may differentiate NASH from bland steatosis. ELF score, calculated from an algorithm incorporating a panel comprised of hyaluronic acid (HA), amino-terminal propeptide of type III collagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) has been

shown to have reasonable predictive capability in adult NASH. Nobili et al assessed ELF score utility in predicting liver fibrosis in 112 children with NAFLD [13]. Area under the receiver operating characteristics curve for diagnosing advanced fibrosis (stage 3 or more) was 0.99 at an ELF cut-point of 10.51. Larger and more diverse groups are needed to validate the diagnostic test characteristics of ELF in pediatric fatty liver disease.

### **Conclusion**

It is imperative to distinguish simple steatosis from NASH since the latter has a progressive disease course and can lead to end-stage liver disease. Liver biopsy has been considered as the gold standard for the diagnosis of NASH. However, liver biopsy is invasive, costly, and can rarely cause significant morbidity (risk of morbidity, 0.06-0.35%; risk of mortality, 0.1-0.01%). Imaging studies such as ultrasonography, computed tomography, and magnetic resonance imaging have limited sensitivity in detecting steatosis and cannot distinguish steatosis from NASH. Alanine aminotransferase (ALT) has been used as a surrogate marker for liver injuries. However, ALT is not an ideal marker for either diagnosis of NAFLD or distinguishing steatosis from NASH. Better noninvasive biomarkers or panels of biomarkers that are cheaper, reliable, and reproducible are urgently needed for patients with NASH to assist in establishing diagnosis, providing risk information, and monitoring disease progression and treatment response [14-17].

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