Histological Abnormalities in Children with Nonalcoholic Fatty Liver Disease and Normal or Mildly Elevated Alanine Aminotransferase Levels

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Objective To investigate the histological spectrum of nonalcoholic fatty liver disease (NAFLD) in children with normal, mildly elevated (26-50 U/L boys, 23-44 U/L girls), or elevated (>50 U/L in boys, >44 U/L in girls) serum alanine aminotransferase (ALT) levels.

Study design The Nonalcoholic Steatohepatitis Clinical Research Network enrolls children aged 5-18 years with NAFLD. We analyzed baseline clinical and histological data from 91 children with suspected NAFLD and normal or mildly elevated ALT and liver biopsy analysis within 180 days of ALT measurement, and compared them with data from 392 children with elevated ALT.

Results Seventeen of the 91 children with suspected NAFLD (19%) had a normal ALT level, and 74 (81%) had a mildly elevated ALT level. Overall, 45% of the biopsy specimens analyzed had steatosis ≥33%, 22% had grade ≥2 lobular inflammation, 81% had portal inflammation, 29% had ballooned hepatocytes, 35% had "suspicious/borderline" steatohepatitis, 8% had definite nonalcoholic steatohepatitis, 34% had an NAFLD activity score ≥4, and 46% had fibrosis (38% mild/moderate and 8% bridging/cirrhosis). Marked steatosis (50% vs 24%) and fibrosis (54% vs 12%) were significantly more common in the patients with mildly elevated ALT compared with those with normal ALT, with no difference in ballooning, inflammation, or NAFLD activity score ≥4 between the 2 groups. Fibrosis stage 3/4 was seen in none of the children with normal ALT, in 9% of those with mildly elevated ALT, and in 15% of those with elevated ALT.

Conclusion Liver biopsy specimens from children with NAFLD with normal or mildly elevated ALT levels show significant histological abnormalities, including advanced fibrosis in children with mildly elevated ALT. Thus, measurement of ALT may underestimate liver injury in NAFLD. The use of appropriate ALT cutoff levels can help identify children at risk for more severe disease. *(J Pediatr 2014;164:707-13)*.

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Nonalcoholic fatty liver disease (NAFLD) is one of the most common comorbidities associated with pediatric obesity. Nonalcoholic steatohepatitis (NASH) can result in cirrhosis, even in childhood.[1-3] American Academy of Pediatrics guidelines recommend biannual measurement of serum alanine aminotransferase (ALT) to screen overweight and obese children for NAFLD.[4] Elevated liver enzyme levels are common in these children.[5] Data regarding an appropriate ALT cutoff level to identify obese children at increased risk for NASH are limited, however.

Upper limit of normal (ULN) ALT values used in US children's hospitals vary widely and lack sensitivity for detecting NAFLD in children. Nationally, the median ULN for ALT is 53 U/L and ranges from 30 to 90 U/L.[6] Recently, new

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
GGT	Gamma glutamyl transpeptidase
HOMA-IR	Homeostasis model assessment of insulin resistance
NAFLD	Nonalcoholic fatty liver disease
NAS	Nonalcoholic fatty liver disease activity score
NASH	Nonalcoholic steatohepatitis
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
ULN	Upper limit of normal

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evidence-based standards for normal ALT levels in children of ≤25 U/L for boys and ≤22 U/L for girls were proposed based on analysis of National Health and Nutrition Examination Survey III data including only children with no risk factors for underlying liver disease.[6]

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) NASH Clinical Research Network (NASH CRN) maintains a large prospective database of clinical information on children with fatty liver.[7] We evaluated clinical and histological variables in children with normal and mildly elevated ALT levels to determine the spectrum of histological abnormalities and assess the usefulness of ALT measurement in identifying active liver disease. A comparison group of children with elevated ALT levels was examined as well.

Methods

The pediatric NAFLD studies were designed by subcommittees of the NASH CRN Steering Committee, composed of principal investigators from each clinical site, the 2 co-chairs of the Pathology Committee, the principal investigator from the Data Coordinating Center, and the NIDDK scientific officer. After approval by the Steering Committee, the study was approved by the Institutional Review Board at each site. Enrolled patients and their guardians provided written informed assent and consent. The protocols, assent/consent forms, and operations manual were approved by a Data and Safety Monitoring Board established by the NIDDK specifically for the NASH CRN. The study was conducted in compliance with Good Clinical Practice Guidelines for Human Research Quality Standards.

Children aged 2-18 years with definite or suspected NAFLD meeting the eligibility criteria were enrolled into the NIDDK's NASH CRN NAFLD Database Study or Pediatric Database 2 Study, 2 observational studies conducted at 12 US medical centers.[8] For the initial database study, inclusion criteria defined NAFLD as a suggestive radiographic study or local liver biopsy evidence of NAFLD. After 2010 (Pediatric Database 2 Study), only children who had been previously enrolled or had undergone standard-of-care liver biopsy within the 3 months before enrollment were eligible. Exclusion criteria for the NASH CRN studies included a history of alcohol consumption, evidence of other forms of chronic liver disease, use of medications known to cause fatty liver, history of total parenteral nutrition, biliopancreatic diversion or bariatric surgery, short bowel syndrome, suspected or confirmed hepatocellular carcinoma, known HIV positivity, conditions likely to interfere with study follow-up, and inability to provide informed consent. Comprehensive data, including demographic data, anthropometric data, medical history information, and routine laboratory study results, were collected for each patient at study entry.

Data included in the present analysis were from children aged 5-18 years enrolled between October 2004 and January 2013 in either of the NAFLD database studies who had histology data from a centrally reviewed biopsy specimen obtained within 6 months of baseline ALT measurement, and whose ALT value measured close to biopsy was normal (≤25 U/L for boys and ≤22 U/L for girls) or mildly elevated (≤26-50 U/L for boys and ≤23-44 U/L for girls). A comparison group consisted of children with elevated ALT (>50 U/L for boys and >44 U/L for girls) and liver biopsy analysis data.

Sample Analysis

Routine laboratory studies were performed on fresh samples in Clinical Laboratory Improvement Amendments-certified laboratories at the clinical sites according to standard clinical protocols. Liver biopsy specimens were fixed in formalin and embedded in paraffin, and unstained slides were sent to the Data Coordinating Center for inclusion in a central pathology repository. Hematoxylin and eosin, Masson trichrome, and Perls iron stains were prepared and reviewed centrally by the NASH CRN Pathology Committee, a group of 9 hepatopathologists blinded to the clinical data. Biopsy specimens were scored by consensus at Pathology Committee meetings based on the NAFLD activity score (NAS) and fibrosis stage.[9] The NASH CRN Pathology Committee designed and validated a histological feature scoring system that addresses the full spectrum of NAFLD lesions for use in clinical trials. This scoring system comprises 14 histological features, with 4—steatosis (score of 0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2), and fibrosis (0-4)—evaluated semiquantitatively and the others recorded as either present or absent. The following diagnoses were rendered: definite steatohepatitis, borderline 1a (centrilobular accentuation) or 1b (portal accentuation), and definitely not steatohepatitis. The NAS was determined by summing the unweighted scores for steatosis, lobular inflammation, and hepatocellular ballooning (total score, 0-8).

Data Analyses

We performed cross-sectional analyses of demographic factors, including sex, age, race (white vs nonwhite) and ethnicity; anthropometric data, including Tanner stage (stage 1 vs other) and body mass index (BMI) *z*-score; and a history of comorbid conditions, including diabetes, hyperlipidemia, and hypertension (reported diagnosis or on current treatment). We also examined serum levels of aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), triglycerides, total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, hemoglobin A1c, fasting glucose, and insulin, as well as the homeostasis model assessment of insulin resistance (HOMA-IR) index. Histological features analyzed included fibrosis stage (none, mild/moderate [1-2], or bridging/cirrhosis [3-4]), NAS, presence of definite or borderline NASH, steatosis grade, lobular inflammation (<2 or ≥2), portal inflammation (none, mild, or greater), hepatocellular