

Original Article



OPEN ACCESS

Received: May 1, 2021

1st Revised: Jul 6, 2021

2nd Revised: Aug 11, 2021

Accepted: Aug 29, 2021

Correspondence to









Asuman Nur Karhan

Department of Pediatrics, Division of
Gastroenterology, Mersin University Faculty
of Medicine, Çiftlikköy Kampüsü, Yenişehir,
Mersin 33110, Turkey.
E-mail: asunurkar83@gmail.com

Copyright © 2021 by The Korean Society of
Pediatric Gastroenterology, Hepatology and
Nutrition

This is an open-access article distributed
under the terms of the Creative Commons
Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>)
which permits unrestricted non-commercial
use, distribution, and reproduction in any
medium, provided the original work is properly
cited.

ORCID iDs

Asuman Nur Karhan 
<https://orcid.org/0000-0003-1665-4255>
Hayriye Hizarcioglu-Gulsen 
<https://orcid.org/0000-0003-1181-7393>
Ersin Gumus 
<https://orcid.org/0000-0002-2280-9789>
Zuhal Akçören 
<https://orcid.org/0000-0001-7108-6129>
Hülya Demir 
<https://orcid.org/0000-0001-9271-7639>
İnci Nur Saltik-Temizel 
<https://orcid.org/0000-0002-5580-5100>
Diclehan Orhan 
<https://orcid.org/0000-0003-3637-5392>
Hasan Özen 
<https://orcid.org/0000-0002-9063-3893>

Distinctive Features of Hepatic Steatosis in Children: Is It Primary or Secondary to Inborn Errors of Metabolism?

Asuman Nur Karhan ¹, Hayriye Hizarcioglu-Gulsen ², Ersin Gumus ²,
Zuhal Akçören ³, Hülya Demir ², İnci Nur Saltik-Temizel ², Diclehan Orhan ³,
and Hasan Özen ²

¹Department of Pediatrics, Division of Gastroenterology, Mersin University Faculty of Medicine, Mersin, Turkey

²Department of Pediatrics, Division of Gastroenterology, Hacettepe University Faculty of Medicine, Ankara, Turkey

³Department of Pediatric Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey

ABSTRACT

Purpose: The incidence of hepatic steatosis among children has been increasing; however, data distinguishing simple steatosis from a more complex disorder are lacking.

Methods: This study identified the etiologies resulting in hepatic steatosis through a retrospective review of pediatric liver biopsies performed in the last 10 years. A total of 158 patients with hepatic steatosis proven by histopathological evaluation were enrolled in the study, and baseline demographic features, anthropometric measurements, physical examination findings, laboratory data, ultrasonographic findings, and liver histopathologies were noted.

Results: The two most common diagnoses were inborn errors of metabolism (IEM) (52.5%) and nonalcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) (29.7%). The three most common diseases in the IEM group were glycogen storage disorders, Wilson's disease, and mitochondrial disease. The rates of consanguineous marriage (75.6%; odds ratio [OR], 26.040) and positive family history (26.5%; OR, 8.115) were significantly higher ($p=0.002$, $p<0.001$, respectively) in the IEM group than those in the NAFLD/NASH group. Younger age ($p=0.001$), normal anthropometric measurements ($p=0.03$), increased aspartate aminotransferase levels ($p<0.001$), triglyceride levels ($p=0.001$), and cholestatic biochemical parameters with disrupted liver function tests, as well as severe liver destruction of hepatic architecture, cholestasis, fibrosis, and nodule formation, were also common in the IEM group.

Conclusion: Parents with consanguinity and positive family history, together with clinical and biochemical findings, may provide a high index of suspicion for IEM to distinguish primary steatosis from the consequence of a more complex disorder.

Keywords: Hepatic steatosis; Metabolism, Inborn errors; Nonalcoholic hepatic steatosis; Pediatric

Conflict of Interest

The authors have no financial conflicts of interest.

INTRODUCTION

Hepatic steatosis, defined as lipid accumulation in the liver parenchyma, is considered an important cause of hepatopathy from infancy to adulthood because it may cause hepatic fibrosis and eventually cirrhosis when uncontrolled [1]. Children with hepatic steatosis may be asymptomatic or present with elevated transaminase levels or various symptoms related to underlying diseases [2,3].

Obesity-related nonalcoholic fatty liver disease (NAFLD) is the most recognized as well as the leading cause of hepatic steatosis globally. However, in children, before the diagnosis of NAFLD, secondary reasons for hepatic steatosis, such as viral hepatitis, inborn errors of metabolism (IEM), celiac disease, autoimmune hepatitis, hepatotoxic drug consumption, malnutrition, and parenteral nutrition, need to be excluded [4-6]. Physicians should especially be suspicious about IEM, even if no disease-specific signs and symptoms are encountered. Since not all patients with IEM present with acute metabolic symptoms in the neonatal period, diseases may present solely with hepatic steatosis [3]. Timely diagnosis of IEM, such as glycogen storage disorders (GSDs) and Wilson's disease, should be carried out to slow down the progression of liver destruction with dietary modifications or specific treatment options.

Due to its high prevalence, the risk factors of NAFLD, including obesity, insulin resistance, and hypertriglyceridemia, have been well described [7]. In those studies, male sex, higher alanine aminotransferase (ALT) levels than serum aspartate aminotransferase (AST) levels, and vague abdominal pain were the other common features [6,8]. Therefore, data on the secondary causes of hepatic steatosis, particularly IEM-related data, are still insufficient due to the relatively low prevalence of this disease group worldwide. This study was designed to identify the clinical, laboratory, radiological, and histopathological findings of the underlying diseases in hepatic steatosis and obtain some supportive clues through two main diagnostic groups: IEM and NAFLD.

MATERIALS AND METHODS

The pathology report archive system of the Hacettepe University Pediatric Gastroenterology Department was retrospectively reviewed to recruit patients aged 0-18 years who were followed up with histologically proven hepatic steatosis between January 2006 and December 2016. Demographic features, medical histories, anthropometric measurements, physical examination findings, primary diagnosis, laboratory data, ultrasonography (USG) results at the time of biopsy, and histopathological findings were noted. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). The WHO Anthro and AnthroPlus software were used to obtain the BMI Z-scores. Patients with a BMI Z-score $>+2$ standard deviation (SD) were grouped as obese [9]. In children <2 years of age, BMI percentiles and Z-scores for girls and boys from birth to 2 years were already available, but not suitable for screening obese infants; thus, obesity was defined as a weight ≥ 95 th percentile for height [10].

The NAFLD group included patients with fatty infiltration $>5\%$ of the liver through imaging, direct quantification, or histologic estimation. Hepatic steatosis, along with one of the following features—inflammation, with or without ballooning injury to hepatocytes

and fibrosis—was defined as nonalcoholic steatohepatitis (NASH) [11,12]. Biopsy samples with significant histopathological findings, such as destruction of the hepatic architecture, cholestasis, liver fibrosis, and nodule formation that belonged to patients who had nonspecific clinical features with normal metabolic tests were classified as the “undetermined group.” Other diagnoses are also presented in **Table 1**.

The cutoff levels were determined based on National Health and Nutrition Examination Survey as follows: ALT and AST, 25.8 U/L for boys and 22.1 U/L for girls, respectively; gamma-glutamyl transpeptidase (GGT) 9–48 U/L; and alkaline phosphatase (ALP) 44–147 U/L [12,13]. Conjugated hyperbilirubinemia was defined as a serum conjugated bilirubin concentration >1.0 mg/dL if the total serum bilirubin is <5.0 mg/dL or >20% of the total serum bilirubin if the total serum bilirubin is >5.0 mg/dL [14]. Triglyceride (TG) levels above the 95th percentile for age, ≥100 mg/dL for 0–9 years and ≥130 mg/dL for 10–19 years were defined as hypertriglyceridemia [15]. Serum albumin levels ≥3.5 g/dL and international normalized ratio (INR) levels between 0.8 and 1.1 were considered normal.

Liver samples were obtained using ultrasound-assisted percutaneous needle biopsy. Histopathological findings such as hepatic architecture, nuclear hyperglycosylation, the type of steatosis (microvesicular, macrovesicular, and intermediate-size fatty change), inflammation, necrosis, cholestasis, fibrosis, and nodule formation were noted. Hepatic steatosis was defined as macro- or microvesicular fatty infiltration of at least 5% of hepatocytes [5].

Statistical analysis was performed using IBM SPSS Statistics (version 22.0.0; IBM Co., Armonk, NY, USA). *p*-values <0.05 were considered statistically significant. For quantitative data, the median and interquartile range (IQR) for qualitative data frequency and percentage were used. Chi-square or Fischer exact tests were used to compare the frequencies of the categorical variables. For further statistical analysis, either Student's *t*-test or Mann–Whitney

Table 1. Spectrum of diagnoses associated with hepatic steatosis and distribution of patients with IEM

Diagnosis	Value
Sex, F/M	57/101
Age*	4 (1, 11)
IEM	83 (52.5)
Diseases in the IEM group	
Glycogen storage diseases	34 (40.9)
Wilson's disease	17 (20.4)
Mitochondrial diseases	11 (13.2)
Hereditary fructose intolerance	6 (7.2)
Fatty acid oxidation disorder	4 (4.8)
Lipid storage disease	3 (3.6)
Tyrosinemia	3 (3.6)
Methylmalonic acidemia	2 (2.4)
Glutaric aciduria	1 (1.3)
Wolcott-Rallison syndrome	1 (1.3)
Chanarin-Dorfman syndrome	1 (1.3)
NAFLD/NASH	47 (29.7)
Undetermined group	15 (9.5)
Cystic fibrosis	9 (5.7)
Toxic hepatitis	3 (1.9)
Focal nodular hyperplasia	1 (0.7)

Values are presented as number only, median (interquartile range [IQR]), or number (%).

IEM: inborn errors of metabolism, NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis.

*Age at biopsy, summarized as median IQR.

U-test was used to compare groups. Area under the curve (AUC) values were obtained using receiver operating characteristic analysis. For the parameters, the threshold and AUC values were obtained. With threshold values, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were calculated and compared with the normal levels. The institutional review board approved the waiver of informed consent because the study did not adversely affect the rights and welfare of the subjects. This retrospective review was approved by the ethical committee of Hacettepe University Hospital (approval number: GO 17/803-1).

RESULTS

A total of 158 patients diagnosed with histologically proven hepatic steatosis were enrolled in the study. Of these children, 63.9% (n=101) were males and 36.1% (n=57) females. Among the 158 patients, 47 (29.7%) had primary hepatic steatosis (NAFLD/NASH), whereas the remaining 111 (70.3%) had hepatic steatosis secondary to underlying diseases. Of the 111 patients, 74.8% (n=83) had IEM, 11.7% (n=13) had hepatic steatosis secondary to determined diseases, and the remaining 13.5% (n=15) had hepatic steatosis due to an undetermined disease. In total, the two major diagnostic groups associated with hepatic steatosis were IEM (52.5%) and NAFLD/NASH (29.7%). The NAFLD/NASH group included 32 patients with NAFLD (68.1%) and 15 patients with NASH (31.9%). The three most common diseases in the IEM group were GSDs (40.9%), Wilson's disease (20.4%), and mitochondrial disease (13.2%). In the GSD group, 38.2% (n=13) had type 1a, 14.7% (n=5) had type 1b, 17.6% (n=6) had type 3a, 2.9% (n=1) had type 11, and the remaining 9 patients were unclassified. The spectrum of diagnoses associated with hepatic steatosis and the distribution of the patients in the IEM group are listed in **Table 1**.

The two major diagnoses of underlying hepatic steatosis, IEM vs. NAFLD/NASH, were compared based on various parameters.

The median age (IQR) of the study population was 6.1 years (4 months to 18 years). Patients in the IEM group were significantly younger than those in the NAFLD/NASH group (1.5 [0.8-7] vs. 11 [8-14] years, respectively) ($p=0.001$). Since there was a male predominance in our cohort, no difference was detected between the two groups.

The rate of family history was 26.5% in the IEM group and 4.3% in the NAFLD/NASH group. The difference was statistically significant ($p=0.002$; odds ratio [OR], 8.115; 95% confidence interval [CI], 1.814-36.291). The rate of consanguinity was 75.6% in the IEM group and 10.6% in the NAFLD/NASH group. The difference was statistically significant ($p<0.001$; OR, 26.040; 95% CI, 9.064-74.813).

In terms of anthropometric measurements, 37.3% (n=59) of the study population had a BMI Z-score higher than +2 SD. The prevalence of obesity was 68% in the NAFLD group, 80% in the NASH group (in total, 57.6% in the NAFLD/NASH group), and 22.8% in the IEM group. The median BMI Z-score of the NAFLD/NASH group was significantly higher than that in the IEM group (1.6 ± 0.6 vs. 0.98 ± 0.35 ; $p=0.01$). In the IEM group, the normal BMI Z-score prevalence was 51.8%, whereas it was 21.2% in the NAFLD/NASH group. Furthermore, 20 out of 83 patients (24%) in the IEM group and 3 out of 47 patients (6.3%) in the NAFLD/NASH group had a BMI Z-score lower than -2 SD.

Physical examination revealed mild to moderate jaundice in 32 out of 158 patients (20.2%). Jaundice due to conjugated hyperbilirubinemia was found in 27 patients (84%) in the IEM group, with a significant difference observed between the two groups ($p=0.004$). A doll-like face was noted in 14 patients (8.8%) with GSD. Abdominal distention was detected in 12 patients (7.5%) with GSD. Hepatomegaly was noted in 101 patients (66.4%) and splenomegaly in 38 patients (24%) on physical examination and confirmed by USG. The USG evaluation was abnormal in 107 patients (67.7%). The ratio of abnormal USG evaluation (either organomegaly or steatosis) was 62.6% ($n=52$) in the IEM group and 68.1% ($n=32$) in the NAFLD/NASH group, and the difference was not statistically significant ($p=0.382$). Hepatic steatosis on USG was detected in 48.7% ($n=77$) of patients, and 50.6% ($n=39$) were reported to have moderate or severe steatosis according to the degree of liver echogenicity. However, the severity of steatosis on USG did not differ significantly between the two major diagnostic groups ($p=0.486$).

Increased ALT and AST levels were observed in 118 (74.7%) and 120 (76%) patients, respectively. Twenty-one (13.3%) patients had normal levels of ALT or AST, abnormal USG results, abnormal findings on physical examination, or abnormal metabolic test results. The patients were distributed as follows: 7 patients with IEM, 6 patients with NAFLD/NASH, 4 patients with Wilson's disease, 2 patients with cystic fibrosis, and 2 patients in the undetermined group. The comparison of the IEM and NAFLD/NASH groups showed that the median AST, GGT, direct bilirubin (DB), ALP, TG, and INR levels were significantly higher in the IEM group ($p<0.001$, $p<0.001$, $p=0.041$, $p=0.043$, $p<0.001$, and $p<0.001$, respectively). Moreover, serum albumin and high-density lipoprotein (HDL) levels were significantly lower in the IEM group ($p=0.002$). The AST levels were higher than the ALT levels in 39 (83%) patients in the NAFLD/NASH group. The comparison of the NAFLD/NASH and IEM groups based on baseline features and laboratory data are listed in **Table 2**. The risk factors for IEM, listed in **Table 3**, were also analyzed. Age <6 years, BMI <19, AST level >116 U/L, ALP level >312 U/L, GGT level >52.4 U/L, DB level >0.9 mg/dL, albumin level <4.1 mg/dL, and INR level >1.16 were observed to have meaningful PPV and NPV.

Table 2. The comparison of the NAFLD and IEM groups based on age, BMI, and laboratory data

Variable	NAFLD/NASH (n=47)	IEM (n=83)	p-value
Age (yr)	11 (8, 14)	1.5 (0.8, 7)	<0.001
BMI (kg/m ²)	23 (18.3, 28.5)	16.7 (14.7, 19)	<0.001
BMI Z-score	1.9 (0.9, 2.4)	0.98 (0.72, 1.54)	<0.001
ALT level (U/L)	110 (76, 154)	144 (67, 208)	0.632
AST level (U/L)	70 (56, 100)	123 (81, 229)	<0.001
GGT level (U/L)	38 (17, 75)	220 (47, 198)	<0.001
ALP level (U/L)	113 (158, 282)	256 (187, 384)	0.043
Albumin level (mg/dL)	4.6 (4.4, 4.8)	4 (3.4, 4.4)	<0.001
INR level	1.1 (0.9, 1.18)	1.33 (1.1, 1.5)	<0.001
Total cholesterol level (mg/dL)	145 (122, 178)	159 (119, 228)	0.147
Triglyceride level (mg/dL)	130 (87, 156)	168 (124, 407)	<0.001
LDL level (mg/dL)	79 (62, 106)	72 (51, 88)	0.150
HDL level (mg/dL)	44 (41, 54)	36 (24, 50)	0.002
Direct bilirubin level (mg/dL)	0.2 (0.1, 0.4)	0.9 (0.6, 1.4)	0.041

Values are presented as median (interquartile range).

NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis, IEM: inborn errors of metabolism, BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, INR: international normalized ratio, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

Table 3. The analysis of parameters to predict IEM versus NAFLD/NASH

Variable	Threshold	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	p-value
Age	<6	0.833 (0.757–0.893)	82.9 (69.2–92.3)	74.7 (64.0–83.6)	65.1 (51.6–76.9)	88.6 (78.7–94.6)	0.0001
BMI	<19	0.829 (0.753–0.889)	74.4 (59.6–86.0)	78.3 (67.9–86.6)	66.2 (51.7–78.5)	84.4 (74.4–91.7)	0.0001
BMI Z-score	<1.22	0.842 (0.746–0.892)	76.2 (61.1–88.6)	80.2 (69.3–87.5)	67.6 (52.6–79.2)	87.7 (76.2–95.1)	0.0001
AST level	>116	0.685 (0.598–0.764)	85.1 (71.8–93.7)	51.8 (40.6–62.9)	50.0 (38.6–61.4)	86 (73.3–94.2)	0.0001
GGT level	>52.4	0.717 (0.631–0.792)	65 (50.7–79.1)	71.8 (60.1–80.5)	56.4 (42.5–69.7)	78.7 (67.7–97.3)	0.0001
Albumin level	<4.1	0.798 (0.718–0.863)	93.4 (82.1–98.6)	55.4 (44.1–66.3)	53.8 (42.2–65.0)	93.9 (83.1–98.6)	0.0001
INR level	>1.16	0.764 (0.682–0.834)	89.1 (76.4–96.3)	63.9 (52.6–74.1)	57.7 (45.4–69.4)	91.4 (81.0–97.1)	0.0001
Triglyceride level	>173	0.697 (0.610–0.775)	80.0 (66.7–90.8)	50.0 (38.7–61.3)	48.1 (36.7–59.6)	82.0 (68.6–91.4)	0.0001
HDL level	<36	0.664 (0.576–0.745)	85.2 (71.7–93.8)	51.2 (39.9–62.4)	50.0 (38.6–61.4)	85.7 (72.7–94.0)	0.0013
Direct bilirubin level	>0.9	0.714 (0.657–0.787)	68.6 (53.1–82.4)	76.4 (63.3–84.2)	62.8 (50.1–70.2)	79.6 (69.2–93.4)	0.0411

IEM: inborn errors of metabolism, NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis, PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval, BMI: body mass index, AST: aspartate aminotransferase, GGT: gamma-glutamyl transpeptidase, INR: international normalized ratio, HDL: high-density lipoprotein.

The prevalence of macrovesicular, intermediate-size, and microvesicular steatoses on histopathological assessment was 62.7% (n=99), 28.5% (n=45), and 8.9% (n=14), respectively. Macrovesicular steatosis was the most common type in both the IEM and NAFLD/NASH groups. However, intermediate-size steatosis was significantly more common in the IEM group (33.7%) than that in the NAFLD/NASH group (19.1%) ($p=0.04$). In the undetermined group, the prevalence of macrovesicular and microvesicular as well as intermediate-size steatoses was 26.6%, 46.8%, and 26.6%, respectively. Thus, in total, 11 out of 15 (73.3%) patients had microvesicular steatosis in the undetermined group. The prevalence of destruction in hepatic architecture ($p=0.02$), cholestasis ($p=0.003$), fibrosis ($p<0.001$), and nodule formation ($p<0.004$) were significantly more common in the IEM group than that in the NAFLD/NASH group, and the differences were statistically significant.

DISCUSSION

We aimed to identify the etiologies underlying hepatic steatosis and reveal a few diagnostic clues regarding different disease groups, since most studies have only focused on NAFLD. The most striking result to emerge from the data was the remarkably higher prevalence of IEM with a wide variety of disease spectrum. Although there were quite a few studies evaluating the underlying causes of hepatic steatosis, in contrast to our study, Hourigan et al. [1] showed that the prevalence of NAFLD (37%) was higher than that in IEM (9%) among 155 pediatric patients. In Mogahed et al.'s study [16] evaluating only the secondary causes for hepatic steatosis in 66 patients, 46.7% of them had IEM. Although the exact consanguinity rate has not been reported, the authors explained this relatively high IEM prevalence with a high rate of consanguinity in their country. Similar to the suggestion by Mogahed et al. [16], the high prevalence of IEM in our study can be attributed to the high percentage of consanguineous marriages. Moreover, the consanguinity rate in the IEM group (75.6%) was remarkably higher than that in our country (24%), as reported by the Turkey Demographic and Health Survey in 2018 [17,18]. In our study, the risk of having IEM was 26 times higher when parents had consanguineous marriage, and the risk of having IEM was 8.1 times higher when there was a positive family history of liver disease. It can be concluded that, although the lack of consanguinity and family history cannot precisely exclude the diagnosis of IEM, positivity in both may give physicians a high index of suspicion to diagnose IEM.

The median age of the children with IEM was 2.5 years, which was consistent with previous reports, as most IEMs were reported to show typical presentations at an early pediatric age

[1,19]. In our study, the median age of patients with NAFLD/NASH was 10.4 years, with a prevalence of 25.6%, whereas it was 14.0 years with a prevalence of 37%, as reported by Hourigan et al. [1,12]. Consistent with this finding, Schwimmer et al. [20] concluded that the prevalence of NAFLD increases with age, ranging from 0.7% for children ages 2 to 4 years to 17.3% for ages 15 to 19 years. According to the guidelines of the European Society of Paediatric Gastroenterology Hepatology and Nutrition, obesity under 3 years of age does not result in liver steatosis, and NAFLD is less probable in children aged 3-10 years [12]. Hence, hepatic steatosis in children under 10 years of age, especially in infants, should be considered as a probability for IEM.

The prevalence of NAFLD in obese children (34.2%) is higher than that in the pediatric population (7.6%) and may even reach up to 70-80% [21,22]. This observation was supported by our results showing a significantly higher median BMI Z-score in the NAFLD/NASH group. However, in our study, none of the obese patients had a diagnosis of NAFLD/NASH, nor all the patients with a diagnosis of NAFLD/NASH were obese, which was supported by Schwimmer et al. [23]. Taken together, obesity in children, especially those with accompanying transaminitis, should raise the suspicion of NAFLD, yet should be evaluated for secondary reasons related to hepatic steatosis [4,24]. It should also be concluded that in the NAFLD/NASH group, 6.3% of patients were malnourished, and lean patients with high transaminase levels also deserve to be investigated for hepatic steatosis [23,24].

The median AST level was remarkably higher in the IEM group. It could be expected that as a result of more severe liver destruction, patients in the IEM group should have remarkably higher levels of ALT. This relative discrepancy in our data could be attributed to the substantial number of patients with GSD in the IEM group. In particular, muscle involvement in type 3a and 11 GSD patients may lead to a gradual increase in AST levels. In our study, consistent with previous reports, 83.0% of patients had higher levels of ALT than AST in the NAFLD/NASH group, and this finding may be a useful diagnostic clue to clinical suspicion [2,7]. High TG and low HDL levels may also increase the risk of IEM, according to our data. The median levels of GGT, DB, and ALP, which are indicators of cholestatic hepatic injury, were higher in the IEM group and supported by the increased ratio of cholestasis in liver biopsy samples. The median serum albumin levels were lower and INR levels were significantly higher in the IEM group, suggesting a more severe liver injury and supported by liver biopsy samples showing an increased ratio of destruction, fibrosis, and nodule formation. Laboratory tests can be useful to differentiate IEM from NAFLD and should be thoroughly investigated for an initial diagnosis before liver biopsy.

Despite its strong advantages, USG has many limitations in the detection of inflammation and fibrosis and may miss advanced liver disease [25]. In previous studies, the reported sensitivity and specificity of USG in patients with NAFLD were 60-80%, which is similar to our results [26,27]. This insufficient sensitivity and specificity of standard USG in both disease groups should underline the importance of liver biopsy and not rely on a normal USG result in suspected cases.

The most common type of steatosis in our cohort was macrovesicular steatosis (62.7%), which was slightly lower than that reported in the study by Hourigan et al. (86%) [1]. The higher prevalence of IEM in the overall data compared to previous literature could be responsible for the predominance of macrovesicular steatosis [28]. In a more recent comprehensive study by Kristiansen et al. [29], macrovesicular steatosis was suggested to be

a more severe form of steatosis than microvesicular steatosis. It should be concluded that the increased ratio of the destruction of the hepatic architecture and fibrosis formation, together with macrovesicular steatosis, was suggestive of a more advanced liver involvement than a simple steatosis in the IEM group.

The strength of the present study is that it represents the data of a remarkably large patient group of children with hepatic steatosis. Besides, the findings regarding the IEM group, including a variety of diseases, provide a better understanding since many of these diseases are extremely rare worldwide. This study has some limitations, such as its retrospective design, which did not allow us to assess the patients' skinfold thickness as well as the fibrosis scores with transient elastography. Advanced pathological techniques, including electron microscopic evaluation or, more importantly, assessment of the mitochondrial surface area, could not be implemented in all samples for a more definite diagnosis, especially in the undetermined group.

In conclusion, the causes of hepatic steatosis in children vary, with its most common cause being IEM in our study. Although liver biopsy is still the gold standard test to confirm the diagnosis of steatosis, parameters including younger age, parental consanguinity, positive family history, jaundice, hepatomegaly, normal anthropometric measurements, and increased levels of cholestatic biochemical parameters with disrupted liver function tests concomitant with high TG and low HDL levels may provide a high index of suspicion for IEM before biopsy. A thorough investigation of biopsy samples combined with other findings may direct physicians for a definitive diagnosis. Future studies evaluating steatosis with more sensitive biomarkers or radiological methods are needed to avoid invasive investigations such as liver biopsy, especially in pediatric patients.

REFERENCES

1. Hourigan SK, Torbenson M, Tibesar E, Scheimann AO. The full spectrum of hepatic steatosis in children. *Clin Pediatr (Phila)* 2015;54:635-42.
[PUBMED](#) | [CROSSREF](#)
2. Silva GH, Hessel G, Coelho KI, Escanhoela CA. Steatosis of indeterminate cause in a pediatric group: is it a primary mitochondrial hepatopathy? *Sao Paulo Med J* 2011;129:217-23.
[PUBMED](#) | [CROSSREF](#)
3. Alfani R, Vassallo E, De Anseris AG, Nazzaro L, D'Acunzo I, Porfito C, et al. Pediatric fatty liver and obesity: not always just a matter of non-alcoholic fatty liver disease. *Children (Basel)* 2018;5:169.
[PUBMED](#) | [CROSSREF](#)
4. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr* 2017;64:319-34.
[PUBMED](#) | [CROSSREF](#)
5. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis* 2001;21:3-16.
[PUBMED](#) | [CROSSREF](#)
6. Molleston JP, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002;97:2460-2.
[PUBMED](#) | [CROSSREF](#)
7. Marion AW, Baker AJ, Dhawan A. Fatty liver disease in children. *Arch Dis Child* 2004;89:648-52.
[PUBMED](#) | [CROSSREF](#)
8. Rashid M, Roberts EA. Nonalcoholic steatohepatitis in children. *J Pediatr Gastroenterol Nutr* 2000;30:48-53.
[PUBMED](#) | [CROSSREF](#)

9. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1995;854:1-452.
[PUBMED](#)
10. Barlow SE; Expert Committee. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 2007;120 Suppl 4:S164-92.
[PUBMED](#) | [CROSSREF](#)
11. Alisi A, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. J Pediatr 2009;155:469-74.
[PUBMED](#) | [CROSSREF](#)
12. Vajro P, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr 2012;54:700-13.
[PUBMED](#) | [CROSSREF](#)
13. Schwimmer JB. Clinical advances in pediatric nonalcoholic fatty liver disease. Hepatology 2016;63:1718-25.
[PUBMED](#) | [CROSSREF](#)
14. Fawaz R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL, et al. Guideline for the evaluation of cholestatic jaundice in infants: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2017;64:154-68.
[PUBMED](#) | [CROSSREF](#)
15. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 2011;128(Suppl 5):S213-56.
[PUBMED](#) | [CROSSREF](#)
16. Mogahed EA, Sayed AA, Khalifa SE, El-Hennawy A, El-Raziky MS. Causes of secondary non-alcoholic fatty liver disease in non-obese children below 10 years. Eur J Pediatr 2020;179:719-26.
[PUBMED](#) | [CROSSREF](#)
17. Waters D, Adeloje D, Woolham D, Wastnedge E, Patel S, Rudan I. Global birth prevalence and mortality from inborn errors of metabolism: a systematic analysis of the evidence. J Glob Health 2018;8:021102.
[PUBMED](#) | [CROSSREF](#)
18. Hacettepe University Institute of Population Studies. 2018 Turkey demographic and health survey. Ankara: Hacettepe University Institute of Population Studies, 2019.
19. Mak CM, Lee HC, Chan AY, Lam CW. Inborn errors of metabolism and expanded newborn screening: review and update. Crit Rev Clin Lab Sci 2013;50:142-62.
[PUBMED](#) | [CROSSREF](#)
20. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics 2006;118:1388-93.
[PUBMED](#) | [CROSSREF](#)
21. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. PLoS One 2015;10:e0140908.
[PUBMED](#) | [CROSSREF](#)
22. Mencin AA, Lavine JE. Nonalcoholic fatty liver disease in children. Curr Opin Clin Nutr Metab Care 2011;14:151-7.
[PUBMED](#) | [CROSSREF](#)
23. Schwimmer JB, Newton KP, Awai HI, Choi LJ, Garcia MA, Ellis LL, et al. Paediatric gastroenterology evaluation of overweight and obese children referred from primary care for suspected non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2013;38:1267-77.
[PUBMED](#) | [CROSSREF](#)
24. Di Sessa A, Cirillo G, Guarino S, Marzuillo P, Miraglia Del Giudice E. Pediatric non-alcoholic fatty liver disease: current perspectives on diagnosis and management. Pediatric Health Med Ther 2019;10:89-97.
[PUBMED](#) | [CROSSREF](#)
25. Karanjia RN, Crossey MM, Cox IJ, Fye HK, Njie R, Goldin RD, et al. Hepatic steatosis and fibrosis: non-invasive assessment. World J Gastroenterol 2016;22:9880-97.
[PUBMED](#) | [CROSSREF](#)
26. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol 2014;20:7392-402.
[PUBMED](#) | [CROSSREF](#)

27. Esterson YB, Grimaldi GM. Radiologic imaging in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Clin Liver Dis 2018;22:93-108.
[PUBMED](#) | [CROSSREF](#)
28. Aly FZ, Kleiner DE. Update on fatty liver disease and steatohepatitis. Adv Anat Pathol 2011;18:294-300.
[PUBMED](#) | [CROSSREF](#)
29. Kristiansen MNB, Veidal SS, Christoffersen C, Jelsing J, Rigbolt KTG. Molecular characterization of microvesicular and macrovesicular steatosis shows widespread differences in metabolic pathways. Lipids 2019;54:109-15.
[PUBMED](#) | [CROSSREF](#)