Predictors of pediatric non-alcoholic fatty liver disease (NAFLD) in obese children and adolescents: Is serum ALT level sufficient in detecting NAFLD?

Objectives: The prevalence of obesity and related comorbidities is increasing in children and adolescents. This study aimed to specify the prevalence of non-alcoholic fatty liver disease (NAFLD) in obese children and adolescents and to identify the predictive factors associated with NAFLD.

Material and Methods: Obese children and adolescents aged 6 to 18 years were included in the study. The presence and degree of hepatosteatosis were evaluated using liver US. The groups with and without NAFLD were compared in terms of demographic, anthropometric, and biochemical parameters.

Results: One hundred fifty-five obese children and adolescents with a median age of 13.8 (4.93) years (43 males, 131 pubertal) were included in the study. We found that 57.4% of obese cases had NAFLD. In the group with NAFLD, serum ALT level, AST level, HOMA-IR, and triglyceride level were significantly higher (p<0.001, p<0.001, p=0.015, p=0.021, respectively), and serum HDL-C level was significantly lower (p=0.001) compared to the group without NAFLD. In the binomial logistic regression analysis, age (β=0.213, OR=1.23, p=0.040) and serum ALT level (β=0.047, OR=1.04, p=0.011) were determining factors for hepatosteatosis. The diagnostic accuracy of elevated serum ALT level in detecting NAFLD was found to be 65.8% with a sensitivity of 77.3% and a specificity of 57.3% (+LR 1.81 and -LR 0.40).

Conclusion: The prevalence of NAFLD determined with US in obese children and adolescents was 57.4%. Age and serum ALT level were found to be predictive factors for hepatosteatosis. Increased ALT alone shows insufficient performance in detecting NAFLD.

Keywords: Adolescents, children, hepatosteatosis, non-alcoholic fatty liver, obese, predictors, prevalence.
INTRODUCTION

Pediatric non-alcoholic fatty liver disease (NAFLD) is described as chronic hepatic steatosis in children and adolescents that is not due to secondary causes such as infection, genetic/metabolic disorder, malnutrition, or statogenic drug use. Non-alcoholic fatty liver is defined as the presence of hepatosteatosis in the absence of hepatocellular injury, and non-alcoholic steatohepatitis (NASH) is defined as hepatosteatosis associated with inflammation and hepatocellular injury. [1,2] NAFLD is a relatively benign condition, but it may progress to end-stage liver disease because of oxidative stress and inflammation. [3]

Non-alcoholic fatty liver disease is mostly associated with obesity, insulin resistance, diabetes, and dyslipidemia. [4,5] With the dramatic increase in childhood obesity, NAFLD is currently one of the most important health problems. As is known, the prevalence of obesity-related comorbidity increases in parallel to obesity. [6] In a meta-analysis study published in 2015, the prevalence of NAFLD was shown to be 34.2% in the clinically obese population and 7.6% in the general population in children and adolescents aged 1 to 19 years. [7] In the USA, NAFLD is the most common cause of chronic liver disorder in children and adolescents. [1]

Because NAFLD is often asymptomatic, screening for NAFLD in obese children and adolescents is more important. [1,4,7] Unfortunately, there is currently no consensus on screening strategies for NAFLD in children and adolescents who are at risk. While the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommends NAFLD screening with serum alanine aminotransferase (ALT) (ALT >45 IU/L) and abdominal US, the North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) recommends NAFLD screening with ALT level (ALT >62 IU/L for boys and ALT >44 IU/L for girls). In the consensus statement of the European Society of Endocrinology and the Pediatric Endocrine Society (ESPE) published in 2017, it was recommended that children and adolescents with a body mass index (BMI) ≥ the 85th percentile should be evaluated with ALT (ALT >25 U/L for boys and ALT >22 U/L for girls) in terms of NAFLD. [8] Although liver biopsy is the gold standard for the diagnosis of NAFLD, its use in children is limited. Therefore, biochemical markers and abdominal US in NAFLD screening will help clinicians in making decisions. [9]

In obese children and adolescents, measurements of body composition such as BMI and trunkal fat index, fasting glucose, fasting insulin, ALT, γ-glutamyltransferase (γGT), uric acid levels and dyslipidemia have been reported to be predictors of NAFLD. [2,7,9-11] This study aimed to specify the prevalence of NAFLD in obese children and adolescents with US and to determine predictive factors for NAFLD. In addition, it was also aimed to compare the diagnostic accuracy of serum ALT level with the NAFLD screening results specified with liver US.

MATERIAL AND METHODS

Children and adolescents aged between 6 and 18 years, who were diagnosed as having obesity at the pediatric endocrinology outpatient clinic between July 1, 2022, and June 31, 2023, were evaluated retrospectively. Data related to age, anthropometric measurements, pubertal status, biochemical parameters, and presence of hepatosteatosis according to US results were recorded from the files of the subjects whose BMI values were above the 95th percentile for age. The subjects who had congenital anomalies, syndromic obesity, endocrine disorders, psychiatric disorders, and diseases known to cause steatosis (viral hepatitis, a history of parenteral nutrition, drug use, and autoimmune and metabolic liver diseases) were not included in the study.

In all subjects, physical examination and pubertal staging were performed by the same pediatric endocrinologist (YÖ). Weight measurements were performed using a calibrated 100 g sensitivity digital scale, and height measurements were performed using a wall-mounted stadiometer (SECA, model 220, Hamburg, Germany). The body mass index was computed by dividing weight by the square of height (kg/m²). Obesity was defined as a BMI value of > the 95th percentile according to the reference curves prepared for the Turkish children and adolescents. [12] This study was approved by the Ordu University Clinical Researches Ethics Committee (2023/177), and informed consent was not required due to the retrospective design of the study. The study was conducted following the ethical guidelines set forth in the Declaration of Helsinki, ensuring the ethical treatment of human subjects in medical research.

Following a fasting period of 8–10 hours, serum glucose, ALT, aspartate aminotransferase (AST), and lipid profile were evaluated with routine standard enzymatic methods using the Roche Cobas 8000 c 702 device. An ALT level >25 IU/ml in boys and an ALT level >22 IU/ml in girls was considered abnormal. [13] Insulin resistance was determined by applying the Homeostasis Model Assessment (HOMA-IR) formula, which involves the product of fasting insulin concentration (U/mL) and fasting glucose concentration (mg/dL), divided by 405. [14]

Serum total cholesterol (TC) levels over 200 mg/dL and triglyceride levels over 100 mg/dL in subjects aged between 6 and 9 years, triglyceride levels over 130 mg/dL in subjects aged between 10 and 19 years, low-density lipoprotein-cholesterol (LDL-C) levels over 130 mg/dL, or high-density lipoprotein-cholesterol (HDL-C) levels under 40 mg/dL were accepted as dyslipidemia. [6] Liver US was performed using Toshiba Alpio 500 by the same radiologist in all patients. The group with NAFLD was classified as grade 1 (mild), grade 2 (moderate), and grade 3 (marked) hepatic steatosis according to liver echo pattern. [9]

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences, version 21.0 (IBM Inc., Chicago, Ill, USA). Normal data distribution was tested with the Kolmogorov-Smirnov test. Descriptive statistics for categorical variables were presented as frequencies and percentages. The continuous variables were displayed as mean±standard deviation or median [interquartile range (IQR)] according to their distribution. The Student T-test (for two groups) or one-way variance analysis (for three or more groups) was used for comparison of the normally distributed continuous variables. The continuous variables without normal distribution were compared using the Mann-Whitney U test or the Kruskal-Wallis test. The categorical variables were compared with the Chi-square test. Spearman correlation analyses were used for correlations between the grade of NAFLD and other parameters. For multivariate analysis, binomial logistic regression analysis was used to evaluate the independent predictive factors of NAFLD. A p-value of <0.05 was considered significant.
RESULTS

One hundred fifty-five obese children and adolescents (43 boys, 131 pubertal) with a median age of 13.8 (4.93) years were included in the study. Hepatosteatosis was found in 89 patients (57.4%) on US examination. Sixty-three patients (70.8%) had grade 1 hepatosteatosis, 19 (21.3%) patients had grade 2 hepatosteatosis, and 7 patients (7.9%) had grade 3 hepatosteatosis. Alanine aminotransferase was found to be increased in 66 patients (42.8%) who were obese. At least one abnormal lipid concentration was found in 59.4% (n=92) of the obese subjects; 40% (n=60) of the patients had hypertriglyceridemia, 12.3% (n=19) had hypercholesterolemia, 25.2% (n=38) had increased LDL-C, and 10.6% (n=16) had decreased HDL-C.

When NAFLD and non-NAFLD were compared, age and pubertal status were not found to be different (p=0.171; p=0.584, respectively). NAFLD was more common in boys compared to girls (76.7% vs. 50%, p=0.003). Weight SDS, height SDS, and BMI SDS were indifferent between the NAFLD group and non-NAFLD group. In the NAFLD group, serum ALT, AST, and triglyceride levels, as well as HOMA-IR, were significantly higher (p<0.001, p<0.001, p=0.015, p=0.021, respectively), and serum HDL-C level was significantly lower (p=0.001) compared to the non-NAFLD group. In the non-NAFLD group, 25.8% (n=17) had increased ALT levels. In contrast, the NAFLD group exhibited a higher prevalence, with 59.6% (n=53) having increased ALT levels (p=0.001).

A comparison of the data between the NAFLD and non-NAFLD groups is shown in Table 1.

When the patients with NAFLD were compared according to the grade of hepatosteatosis, serum ALT level was found to be significantly higher in grade 2 and grade 3 compared to grade 1 (p=0.006 and p=0.013, respectively), and serum AST level was found to be significantly higher in grade 3 compared to grade 1 (p=0.019). Serum fasting insulin level was found to be significantly higher in grade 3 compared to grade 1 and grade 2 (p=0.008 and p=0.037, respectively), and HOMA-IR was found to be significantly higher in grade 3 compared to grade 1 (p=0.007). A comparison of the data between the three patient groups with grade 1, grade 2, and grade 3 hepatosteatosis is shown in Table 2.

In the binomial logistic regression analysis, it was observed that the model was statistically significant (Pseudo R²=0.25, p<0.001). In the binomial logistic regression analysis, age (β=0.213, OR=1.23, p=0.040) and ALT value (β=0.047, OR=1.04, p=0.011) were found to be statistically significant determining factors in detecting NAFLD (Table 3).

A positive correlation was found between the degree of hepatosteatosis and weight SDS, BMI SDS, fasting insulin, HbA1c, HOMA-IR, ALT, and AST (p<0.001, r=0.454; p<0.001, r=0.372, respectively). A negative correlation was found...
A significant correlation was found between the degree of hepatosteatosis and HDL (p<0.001, r=-0.265). The correlations between the degree of NAFLD and other parameters are shown in Table 4.

Based on the ROC curve, the cut-off value for ALT to predict hepatosteatosis was found to be 26 IU/L, with a sensitivity of 56.8% and a specificity of 79.4% (AUC=0.723, p<0.001). The ROC curve for serum ALT level to predict NAFLD is shown in Figure 1.

Alanine aminotransferase was found to be increased in 51 patients (57.3%) who were found to have hepatosteatosis on liver US. The diagnostic accuracy of an increased ALT level in detecting hepatosteatosis was found to be 65.8% with a sensitivity of 77.3% and a specificity of 57.3% (positive likelihood ratio 1.81 and negative likelihood ratio 0.40). Increased ALT showed insufficient performance in detecting hepatosteatosis.
In this study, the data obtained from 155 children and adolescents aged 6 to 18 years were used to determine the predictive factors of pediatric NAFLD. Using liver US, we found that 57.4% of obese children and adolescents had NAFLD. In the binomial logistic regression analysis, age and ALT level were found to be predictive factors for NAFLD. However, use of ALT level alone showed insufficient performance in detecting NAFLD.

<table>
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SDS: Standard deviation score; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FBG: Fasting blood glucose; HbA1c: Haemoglobin A1c; HOMA-IR: Homeostatic model assessment of insulin resistance; LDL-C: Low-density lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol.
Drawing upon earlier research findings, the prevalence of non-alcoholic fatty liver disease (NAFLD) in obese children and adolescents has been reported to range from 34.7% to 70.7%.[10,11,15–24] These prevalence rates are compatible with the NAFLD prevalence we found in our study. Considering the increase in the prevalence of obesity, it is inevitable that the prevalence of NAFLD will increase further in the future.[25]

In studies conducted up to now, age,[10] gender,[19] BMI,[10,16,19] ALT,[10,16] γGT,[3] uric acid,[3,10] glucose,[10] insulin,[10] HOMA-IR,[23] triglyceride,[16,20,26] LDL-C,[11] and HDL-C[16] have been reported to be predictive factors of NAFLD in the pediatric obese population. Discrepancies in study outcomes may be attributed to variations in the age composition of study populations, the inclusion of overweight children, and the utilization of different screening methods to assess NAFLD.

It is known that age, sex, and puberty have different effects on the development of NAFLD.[15,26,27] In our current study, no significant differences were observed in age and pubertal status when comparing subjects with and without NAFLD; however, boys exhibited a higher prevalence of NAFLD. This finding aligns with previous studies reporting a higher prevalence of NAFLD in male populations.[3,10,19,23,26,28] The most important cause of a higher prevalence of NAFLD in males is the fact that the possibility of obesity is higher in males compared to females.[25]

In our study, the lack of a significant difference in terms of pubertal status between individuals with and without NAFLD may be attributed to the predominant inclusion of participants in pubertal age groups. Increasing NAFLD prevalence with advancing age was associated with a longer disease period from the beginning of obesity. Another factor contributing to age difference is the role of puberty in NAFLD development.[26] Suzuki et al.[27] claimed that insulin resistance developing with pubertal maturation might be explained by the change in estrogen levels during puberty in both boys and girls. Akcam et al.[19] demonstrated that there was a strong relationship between NAFLD liver US scores and HOMA-IR. The relationship between insulin sensitivity and the degree of NAFLD may be evidence suggesting that insulin resistance may cause fat accumulation in hepatocytes in puberty.[15]

ESPGHAN recommends liver function tests and liver US for NAFLD screening in obese children,[8] whereas NASPGHAN recommends ALT as a screening test. Measuring serum ALT levels is considerably more cost-effective compared to imaging methods, and it is recommended as the primary screening test because it is minimally invasive. However, liver function tests used for screening NAFLD have significant limitations.[1] The cut-off value for serum ALT level to detect liver disease in children is controversial. In the Screening ALT for Elevation in Today's Youth (SAFETY) study, it was demonstrated that the upper threshold values of normal for ALT used in children were too high to detect chronic liver diseases.

According to data from the National Health and Nutrition Examination Survey (NHANES), the 95th percentile values for ALT in healthy pediatric participants were 25.8 U/L for males and 22.1 U/L for females. When current hospital ALT threshold values (53 U/L) were used, the sensitivity for detecting NAFLD was found to be 32% for the boys and 48% for the girls; the specificity was found to be 92% for the boys and 96% for the girls. When the threshold values derived from NHANES were used, the sensitivity was found to be 72% for the boys and 85% for the girls; the specificity was found to be 79% for the boys and 85% for the girls.[13]

In a study in which overweight and obese children, who were referred from primary care to pediatric gastroenterology, were evaluated, a diagnosis of NAFLD was made in 43% of the subjects who had an ALT level of 40–80 and in 81% of the subjects whose screening ALT level was ≥80. In this study, an ALT level of ≥80 had a sensitivity of 57% and a specificity of 71% for the diagnosis of NAFLD. In addition, it was shown that all overweight and obese children with a positive ALT screening result would not necessarily have liver disease, and might have liver disease other than NAFLD or advanced fibrosis.[29]

In other studies, significant histological abnormalities such as advanced fibrosis were shown in some of the children with NAFLD who had normal or slightly elevated ALT levels. Therefore, measurement of ALT alone may underestimate the degree of liver injury in NAFLD.[30] In addition, NAFLD screening based on ALT value alone may miss NAFLD.[7] In our study, ALT was found to be increased in 57.3% of the patients who were found to have NAFLD in liver US (ALT >25 in boys and ALT >22 in girls). The ALT cut-off value for NAFLD specified with ROC analysis (26 IU/L) was similar to the value recommended by Schwimmer et al.[13] to be used in detecting chronic liver diseases in children. However, the diagnostic accuracy of increased ALT alone was found to be 65.8%, which represented an insufficient performance in detecting NAFLD.

Ultrasonography, one of the routine imaging methods, is commonly used to identify the presence of hepatic steatosis. However, the positive predictive value of liver US for hepatosteatosis was found to be 47%–62% in a systematic review evaluating the imaging methods used for assessment of hepatosteatosis in children. Therefore, the use of US for diagnosing or grading NAFLD is not recommended in children.[31] Liver US is a noninvasive, safe, and inexpensive tool for the diagnosis of NAFLD, though it has limitations. In many studies, it was shown that liver US had benefits in the diagnosis of NAFLD.[7,26,32] Since US is not able to differentiate simple steatosis from non-alcoholic steatohepatitis accurately, it is only reliable when hepatic steatosis is moderate or severe, and it cannot assess the severity of fibrosis reliably.[26]

The fact that a correlation could not be shown between increased ALT and US findings in children demonstrated that measurement of liver enzymes alone for NAFLD screening was not sufficient. Therefore, screening with liver US is recommended to detect NAFLD in the early phase.[15] A screening strategy that used the combination of increased ALT and fatty infiltration on US was shown to increase the sensitivity for the detection of NAFLD. Therefore, pediatric NAFLD screening panels that combine ALT with waist circumference and other metabolic data have been developed to increase screening sensitivity.[33]

It is known that the prevalence of dyslipidemia is high in obese children.[20,23] Due to the association between visceral adiposity and NAFLD, there is an excessive release of free fatty acids into
the bloodstream resulting from lipolysis, along with increased hepatic glucose production, leading to an increase in peripheral insulin resistance. Akcam et al.[18] reported that insulin resistance may adversely impact fat accumulation in the hepatocytes in puberty owing to a strong relationship between hepatosteatosis scores on liver US and HOMA-IR.

Free fatty acids released excessively because of lipolysis in obesity lead to hypertriglyceridemia by increasing the production of very low-density lipoprotein (VLDL) and TG in the liver and inhibiting lipoprotein lipase in fat and muscle tissues.[22] Therefore, the most commonly reported dyslipidemia in obesity is hypertriglyceridemia.[3,18,22,23,26] In a study conducted by Elmaoğulları et al.,[22] the prevalence of dyslipidemia was reported to be 42.9% in obese children and adolescents aged 2 to 18 years. In the same study, the association of dyslipidemia and hepatosteatosis was found to be correlated with higher HOMA-IR levels and abnormal liver function test results.[22]

In the current study, the prevalence of dyslipidemia was found to be 57.4% in children aged between 6 and 18 years, and 40% of the dyslipidemic subjects had hypertriglyceridemia. The higher prevalence of dyslipidemia in our cohort, possibly arising from the older age of obese children and differences in reference lipid levels, is noteworthy.

The limitations of our study included the fact that it was a single-center retrospective study, that NAFLD was diagnosed subjectively by way of liver US, and that methods evaluating body composition were not used to define obesity. Prospective studies are needed to make comparisons with a control group consisting of children with normal weight in order to determine the predictors of NAFLD in obese children and adolescents.

CONCLUSION

In conclusion, the prevalence of NAFLD is considerably high in obese children and adolescents. Age and ALT level are predictive factors for NAFLD. However, the use of ALT level alone showed insufficient performance in detecting NAFLD. Liver US is an eligible screening method for NAFLD, though it has some limitations.

REFERENCES


