The Relationship Between Lipid Profile and Non-alcoholic Fatty Liver Disease in Children and Adolescents with Obesity

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ABSTRACT

Objective: To determine the relationship between lipid profile and non-alcoholic fatty liver disease in obese children and adolescents.

Study Design: A comparative cross-sectional study.

Place and Duration of Study: Department of Pediatrics, Kastamonu Training and Research Hospital, Turkey, from March 2018 to April 2021.

Methodology: Clinical, laboratory and ultrasound findings of 290 children and adolescents diagnosed with obesity were analysed. Children and adolescents were divided into two groups as those who were diagnosed with NAFLD by ultrasonography (USG) and those who did not were diagnosed. Two groups were compared in terms of clinical and biochemical findings.

Results: The body mass index (BMI), bodyweight for height (BWH), insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), free T4, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), hemoglobin A1c (HbA1c) and HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) levels were found to be significantly higher ($p<0.05$) in the children and adolescents with NAFLD In obese children and adolescents; there was a significant correlation between the increase in TC, LDL-C and TG levels in the blood and the development of NAFLD ($p<0.05$). Blood ALT level ≥37 U/L in obese boys and ≥23.5 U/L in obese girls was found to be a strong diagnostic biomarker in determining the presence of NAFLD.

Conclusion: In obese children and adolescents, there was a significant relationship between the increase in BWH and blood lipid levels, insulin resistance and the development of NAFLD. ALT had high specificity and sensitivity to predict non-alcoholic fatty liver disease.

Key Words: *Obesity, Children, Hepatosteatoz.*

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an abnormal fat accumulation and characterised by more than 5% of hepatocytes without alcohol consumption, drug use, viral infections, metabolic and autoimmune diseases or genetic disease.[1,2] It is the most common chronic liver disease in children and the most common indication for liver transplantation in young adults in the USA.[3] The prevalence of NAFLD has more than doubled in the United States over the past 20 years.[4]

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Although the liver biopsy is the gold standard method in the diagnosis of NAFLD, it is not generally preferred due to the fact that it is invasive and costly.[5] In addition to serum biomarkers such as ALT and AST in overweight or obese children and liver USG is currently the most widely used tool for diagnosis.[6] Besides insulin resistance, obesity and related dyslipidemias, which are featured by an increase in TG and LDL-C and a decrease in high--density lipoprotein cholesterol (HDL-C) are thought to be the most important risk factors for NAFLD.[7]

As can be seen, weight gain and obesity are primary risk factors for the development of NAFLD. Further evidence suggests that diet composition, particularly carbohydrates, has an important role in the progression of the disease to NASH and fibrosis.[8] In other words, nutrition habits are an important starting point for NAFLD. It is predicted that the frequency of obesity and NAFLD will increase due to the increasing malnutrition habits in children and young adults. For this reason, this study aimed to

investigate the relationship between lipid profile and non-alcoholic fatty liver disease in obese children and adolescents, by comparing the clinical and biochemical findings of the children and adolescents with and without NAFLD.

METHODOLOGY

Two hundred and ninety obese children and adolescents who presented to the Pediatrics Clinic between March 2018 and April 2021 and were diagnosed with obesity and a BMI ≥95p for ≥2 years, ≥97.7p of weight-for-height percentile for <2 years of age, fasting blood lipid profile (TC, LDL-C, HDL-C, TG), and who had NAFLD evaluation with abdominal USG.

The patients were evaluated retrospectively and the age (month) closest to the date of USG, gender, body weight (BW), height, BWH, BMI (BW/height ^{2} = Kg/m ^{2}), blood glucose, insulin, ALT, AST, free T4 (fT4), thyroid-stimulating hormone (TSH), TC, LDL-C, HDL-C, non-HDL-C, TG, HbA1c levels and insulin resistance HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) scores were recorded. Non-HDL-C levels were calculated by subtracting HDL-C levels from TC.[9] HOMA-IR score was calculated with the formula of (glucose) x (insulin)/405, and a score >3.42 was considered as IR (+).[10]

The TC, LDL-C, HDL-C and TG levels of the patients were compared with the age and sex-standardized percentile chart,[11] and recorded as low (<5p), normal (5p-95p) and high (>95p). In this way, the patients were divided into three groups according to their blood lipid levels and the biochemical parameters of the groups were compared.

The Statistical Package for Social Sciences-23 (SPSS-23) program was used to analyze the data. The data with normal distribution was checked with the Kolmogorov-Smirnov test. The median and 25-75th percentile values of non-normal continuous variables were calculated. Categorical variables were expressed as numbers and percentages. The significance of the differences between the groups in non-normal variables was evaluated using the Mann-Whitney U test and the Kruskal-Wallis test. Pearson Chi-square test was used to determine the relationship between categorical variables with two or more groups. The optimal cut-off values and area under the curve of continuous ALT and AST variables were calculated by applying the Receiver Operating Curve (ROC) analysis. The p<0.05 value was considered statistically significant.

RESULTS

The mean age of the patients was 12.06 ± 3.3 years. One hundred and twenty-five (43.1%) of these patients were diagnosed with NAFLD. Of the patients diagnosed with NAFLD 52% (n: 65) were girls and 48% (n:60) were boys. Demographic and biochemical findings of patients with and without NAFLD are given in Table I. The values of several parameters (BMI, BWH, insulin, ALT, AST, fT4, LDL-C, TG, non-HDL-C, HbA1c, HOMA-IR) were computed to be higher in patients with NAFLD than in patients without NAFLD. NAFLD was seen in 64.8% of those with IR (+) and the incidence of NAFLD was significantly higher in those with IR (+) than those with IR (-) (p=0.001).

Table I: Demographic and biochemical findings of patients with and without NAFLD.

Variables	With NAFLD (n=125, 43.1%)	Without NAFLD (n=165, 56.9%)	p
Gender (female/male)	65/60 (%52/48)	107/58 (%64.8/35.2)	0.027**
Age (years)	11,5 (9,6-15)	12,25 (9,7-15,3)	0.322*
BW (kg)	56 (41-70.50)	55 (39.50-65.50)	0.198*
Height (cm)	148 (135-161)	153 (137.5-160)	0.821*
BMI (kg/m^2)	25.1 (22.2-27.65)	24 (21.05-25.80)	0.001*
BWH (cm)	129 (125-144)	123 (122-126)	<0.001*
Glucose (mg/dL)	88 (84-94)	88 (83-93)	0.314*
Insulin (U/mL)	20 (12-30)	15 (10-22)	0.001*
ALT (U/L)	62 (55.5-69.5)	15 (12-17.50)	<0.001*
AST (U/L)	42 (37-52)	18 (15-22)	<0.001*
fT4 (ng/DL)	1.2 (1.37-1.1)	1.1 (0.9-1.3)	<0.001*
TSH (mIU/mL)	2.8 (2.2-4.1)	2.70 (2.05-3.85)	0.456*
TC (mg/dL)	154 (139-178.5)	148 (132-166)	0.063*
LDL-C (mg/dL)	101 (83.5-121)	94 (80-109.50)	0.007*
HDL-C (mg/dL)	44 (38-49)	46 (39-53)	0.065*
TG (mg/dL)	108 (78.5-155)	91 (70-121.50)	0.016*
Non-HDL-C (mg/dL)	109 (92.5-132)	104 (84-120)	0.009*
HbA1c (%)	5.3 (5.2-5.7)	5.2 (4.9-5.5)	0.001*
HOMA-IR (mg/dL)	4.2 (2.5-6.5)	3.1 (2.1-4.7)	<0.001*

**Mann-Whitney U-test ** Pearson's Chi-square test.*

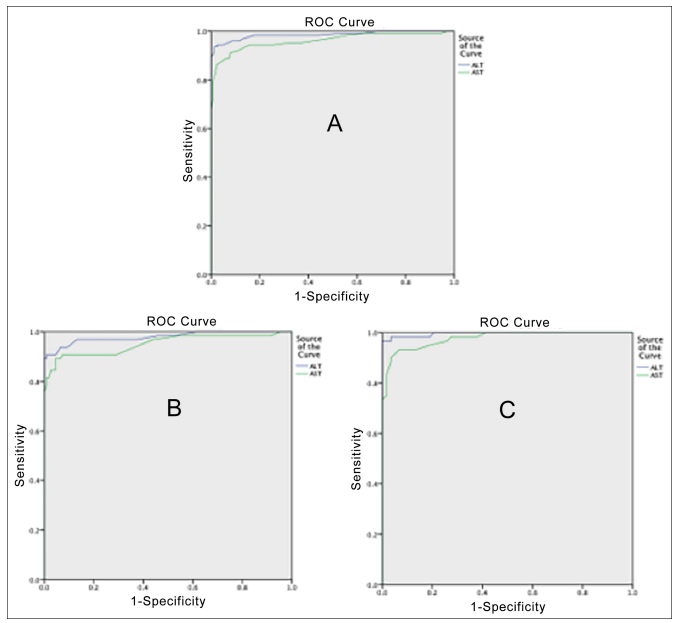

Figure 1: ROC curves of ALT and AST variables for all patients (A), females (B) and males (C) with nonalcoholic fatty liver disease.

The clinical properties and biochemical findings in patients with and without NAFLD by LDL-C levels were compared. Statistically significant differences were found between BWH (p=0.013), ALT (p<0.001), AST (p=0.002), fT4 (p=0.038), TC (p<0.001), TG (p<0.001), non-HDL-C (p<0.001) and HbA1c (p<0.001) in terms of the LDL-C levels. All of the laboratory results were higher in the group with high levels of LDL-C than the other groups (groups with normal and low levels of LDL-C). Moreover, it was higher in the group with normal levels of LDL-C compared to the group with low levels of LDL-C.

The comparison of patients according to their TG levels has been presented in Table II. The parameters (TG, ALT, AST, TC, LDL-C, HDL-C, non-HDL-C, HbA1c) except HDL-C were found to be higher in the group with high levels of TG compared to the groups with normal and low levels of TG. These results were also higher in group with normal level of TG in comparison to group with low level of TG. On the other hand, increasing HDL-C showed a significant decrease in the level of TG.

Table II: Demographic and biochemical findings of patients according to their TG levels.

Variables	TG Level Low n=10, 3.5%	TG Level Normal n=170, 58.6%	TG Level High n=110, 37.9%	p*
Age (years)	11,25(9,81-16)	12.1 (9,6-15,3)	11,6 (9,5-14)	0.419
BW (kg)	56.5 (44.75-65.25)	56 (42-68)	55 (38.75-68.25)	0.869
Height (cm)	153.5 (139.75-157.75)	153 (138-160)	149 (135-158.5)	0.673
BMI (kg/m^2)	25.55 (22.8-25.3)	24.35 (21.9-26.5)	24.55 (21.65-26.5)	0.993
BWH (cm)	128 (124.5-130.5)	124.5 (122-129.25)	125 (122-136.25)	0.226
Glucose (mg/dL)	91.5 (90.25-93.5)	88 (84-93)	88 (82.75-94)	0.260
Insulin (U/mL)	15 (9.25-22.75)	15.5 (10-25)	19 (13-27.25)	0.051
ALT (U/L)	13 (10.75-18.25)	18.5 (14-58)	46 (15-64)	0.002
AST (U/L)	19 (15.5-22)	23 (17-40.25)	28 (18-43)	0.048
fT4 (ng/DL)	1.2 (1.1-1.32)	1.2 (0.9-1.3)	1.2 (1-1.3)	0.389
TSH (mIU/mL)	2.75 (1.92-4.8)	2.6 (2.1-3.75)	3.15 (2.2-4.2)	0.122
TC (mg/dL)	132 (114.5-140.75)	147 (128-161.25)	163 (143.75-190)	<0.001
LDL-C (mg/dL)	68.5 (53.5-82)	94 (80-108)	107 (88.75-129.25)	<0.001
HDL-C (mg/dL)	59 (52.75-67.5)	45 (40-52)	43 (36-48)	<0.001
Non-HDL-C(mg/dL)	76 (58.75-85)	101 (83-116.25)	120.5 (101.5-142.5)	<0.001
HbA1c (%)	5.05 (4.8-5.3)	5.3 (5-5.5)	5.4 (5.1-5.6)	0.012
HOMA-IR (mg/dL)	3.3 (2.03-5.13)	3.2 (2.1-5.63)	3.9 (2.5-6)	0.126

**Kruskal Wallis Test*

Optimal cut-off values of ALT and AST parameters were calculated by ROC (Receiver Operating Characteristic) analysis. The areas under the curve (AUC) of ALT and AST for all patients with NAFLD were 0.986 and 0.962, respectively (Figure 1). The AUC values for ALT and AST were 0.980 and 0.951 in females with NAFLD, and 0.996 and 0.977 in males, respectively. These values showed that ALT and AST are strong diagnostic biomarkers for NAFLD. In addition, the optimal cut-off values for ALT and AST for all patients with NAFLD were 31.5 and 26.5, respectively; 23.5 and 24.5 for females with NAFLD, respectively and for males, it was 37.0 and 29.5, respectively. While 94.4% of all children with NAFLD could be predicted correctly with ALT, 2.4% were predicted incorrectly. In females with NAFLD, the sensitivity of ALT was 93.8% and the specificity was 93.5%; in males, it was 98.3% and 96.6%, respectively. A blood ALT level of ≥37 U/L in obese males and ≥23.5 U/L in obese females was found to be a strong diagnostic biomarker in determining the presence of NAFLD.

DISCUSSION

In this study, the TC, LDL-C, non-HDL-C, TG, insulin, HbA1c and HOMA-IR values were found to be significantly higher in patients with NAFLD compared to patients without. Hazer *et al*. reported similar findings in their study.[11] Jimenez-Rivera *et al*. found that only TG elevation in blood lipids was significant in obese children with NAFLD compared to non-obese children and they reported that the elevation of TC, LDL-C and non-HDL-C was not significant.[12] On the other hand, Öz *et al*. found that a one-unit increase in the HOMA-IR score in obese children increased the risk of NAFLD by 1.35 times.[13] In Tunç's study, significantly higher insulin levels and HOMA-IR were found in the group with NAFLD compared to the group without.[14] In addition, a positive correlation was found between the level of HOMA-IR and the severity of NAFLD. These findings suggest that hyperlipidemia and blood sugar irregularity are important risk factors in the development of NAFLD.

The frequency of dyslipidemia (increased TG and LDL-C levels and decreased HDL-C levels), which is an important risk factor for NAFLD, is increased in obese patients.[15] In this study, as the TG levels of the patients increased, there was a significant decrease in HDL-C levels (dyslipidemia) and the frequency of NAFLD increased. Toledo *et al*. found an increase in serum TG levels and a decrease in HDL-C levels in patients with moderate and severe NAFLD compared to the healthy control group.[16] Nigam *et al*. reported that TC, LDL-C and TG levels were higher and HDL-C levels were lower in patients with NAFLD compared to those without.[17] Şenyiğit *et al*. concluded that there was an increase in serum LDL-C and TG levels, and a decrease in HDL-C levels in the group with NAFLD compared to the group without.[18] These findings support the opinion that dyslipidemia is an important risk factor for NAFLD.

NASPGHAN guidelines recommend ALT as the best screening test for NAFLD in children. These guidelines recommend ALT ≥ 80 U/L on initial screening or twice the upper limit of normal on repeat screening (ALT ≥ 44 U/L for females and ALT ≥ 52 U/L for males).[19] Elizabeth *et al*.[20] reported the optimal cut-off value of ALT for the diagnosis of NAFLD as 30 U/L in females and 42 U/L in males. In this study, the optimal cut-off value of ALT for the diagnosis of NAFLD was found to be lower than the literature, and it was found that it provides good diagnostic accuracy in determining the diagnosis of NAFLD with high sensitivity and specificity.

As a result of the change in the hypothalamo-pituitary axis due to the increased adipose tissue in obese patients, the leptin level increases and the increased leptin causes abnormal TSH secretion. Excess secretion of TSH causes an increase in the amount of fat.[21] Torun *et al*. reported that there was a significant increase in TSH levels as the degree

of the fatty liver increased in obese children, but there was no significant difference in free T3 (triiodothyronine) and fT4 levels.[22] In their study of 332 obese children Kaltenbach *et al*. reported that the TSH level was significantly higher in the NAFLD group than in the non-NAFLD group, and there was no significant difference in T3 and T4 levels.[23] Pacifico *et al*. reported that there was a significant increase in TSH levels in children with NAFLD compared to those without, and there was no significant difference in T3 and T4 levels.[24] These results suggest that obese children with NAFLD may have increased leptin and TSH levels due to increased adipose tissue. In our study, unlike the literature, it was found that the T4 level was significantly higher in children with NAFLD than those without, and there was no significant difference in TSH level.

CONCLUSION

NAFLD continues to be an important cause of morbidity in children and adolescents with its increasing prevalence. High BWH, ALT, TC, LDL-C, non-HDL-C, TG, insulin and HOMA-IR levels were found to be associated with NAFLD. Detection of ALT elevation in routine blood tests in obese children should be a warning for the development of NAFLD.

ETHICAL APPROVAL:
Ethics Committee approval was received from the Ethics Committee of Kastamonu University Faculty of Medicine (No. 2020-KAEK-143-100, dated June 23, 2021).

PATIENTS' CONSENT:
Because this study was retrospective, the condition of patients' consent was waived.

CONFLICT OF INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
EC: Carried out the conception and design of the research, drafted the manuscript, and carried out the analysis and interpretation of data.
FCI: Performed the statistical analysis.
EE, EY: Participated in the acquisition of data.

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