Original Article

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.1190206
J Health Sci Med 2023; 6(1): 35-39

Analysis of clinical findings and serum micronutrients in pediatric patients with nonalcoholic fatty liver disease

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Cite this article as: Keçeli Başaran M, Gürkan O. Analysis of clinical findings and serum micronutrients in pediatric patients with nonalcoholic fatty liver disease. J Health Sci Med 2023; 6(1): 35-39.

ABSTRACT

Aim: Nonalcoholic fatty liver disease (NAFLD) is the hepatic finding of systemic lipid and energy metabolism disorder. NAFLD is frequently observed in people with higher body mass index. Serum micronutrient levels play an important role in hepatic metabolism.

Material and Method: This study included 60 NAFLD and 66 control patients. . NAFLD and control groups were compared in terms of ultrasonography (USG) and shear wave elastography (SWE) results. The two groups were compared in serum lipid profile, aminotransferase, insulin, glucose, and HOMA-IR. Both groups were then analyzed in terms of serum ferritin, B12, and vitamin D levels.

Results: . 35% (n=21) of the patients in the NAFLD group had grade 1, 55% (n=33) had grade 2 and 10% (n=6) had grade 3 adiposity. HOMA-IR and insulin levels were higher in the NAFLD group (p=0.02; p=0.001). While the serum ferritin level of the patients in the NAFLD group was higher than the control group (p=0.001); the B12 level was lower (p=0.006). In terms of vitamin D, there was no difference (p=0.368).

Conclusion: It is essential to identify risk factors in children on follow-up due to NAFLD. USG and liver function tests remain the first option in the diagnosis and screening of NAFLD in children. Serum ferritin, B12, and vitamin D levels of children on follow-up due to NAFLD should be analyzed in consideration of liver fattening.

Keywords: Fatty liver, vitamin, ferritin, elastography

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the hepatic finding of systemic lipid and energy metabolism disorder. Impairment in carbohydrate and lipid metabolism leads to lipid accumulation in hepatocytes. NAFLD is more frequently observed in people with higher body mass index (Body mass index: BMI >30) and certain ethnic groups (1). The term NAFLD is a general terminology covering a wide diagnostic spectrum ranging from simple fatty liver to steatohepatitis. There are two subtypes of NAFLD: nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH). Unlike nonalcoholic fatty liver, NASH is considered a progressive form of the disease (2). NAFLD has been associated with obesity, Diabetes Mellitus (DM), hypertension, and dyslipidemia. Insulin resistance (IR) is also considered an independent risk factor for NAFLD severity. It is also thought to be an initiating factor (3).

The fact that NAFLD is recently observed highly regularly and at increasing frequency suggests that urgent counter-measures are necessary. Following the definition of the disease, it was understood that approximately 40% of patients previously diagnosed with cryptogenic cirrhosis were NAFLD (4). The prevalence of NAFLD is around 75% in obese patients (5). The incidence of NAFLD and NASH is increasing in children and adolescents due to obesity, IR, and metabolic syndrome resulting from a sedentary life (6).

The liver exerts a significant effect in micronutrient metabolism. Disruption of energy and nutrient metabolism causes NAFLD. Lipid accumulation in cells leads to lipotoxicity. Fibrosis may develop with the liver inflammatory and parenchymal cell reaction. It is thought that micronutrients play an important role in this cycle (7). There are studies on antioxidant vitamins A, B, and D in particular (8,9). Iron also has an association with NAFLD. It has been determined that liver lipid peroxidation and damage increase with the rise of iron and ferritin levels in the blood (10).

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Received: 17.10.2022 **Accepted:** 06.11.2022

Vitamin D, another micronutrient, was found to be low in obese and NAFLD patients. It was noted that NAFLD is increased in obese rats with vitamin D deficiency. (11).

Micronutrients act as cofactors and play a key role in the liver synthesis, and inflammatory processes. Studies on micronutrients in children with NAFLD are limited. The objective this study; is a comprehensive evaluation of micronutrients, which are as important as carbohydrate and lipid metabolism, together with clinical, laboratory and imaging findings in children with NAFLD. It is thought that the results obtained in this study will guide the follow-up of children with NAFLD in averting progress and assisting treatment.

MATERIAL AND METHOD

This study is a single-center and cross-sectional study which was conducted with the permission of Gaziosmanpaşa Training and Research Hospital Ethics Committee (Date: 19.08.2020, Decision No: 144). All procedures involving human participants were performed in accordance with 1964 Declaration of Helsinki and subsequent amendments.

Children between the ages of 8 and 18 who applied to the pediatric gastroenterology clinic of Gaziosmanpasa Training and Research Hospital between 2018 and 2020 were admitted to the study. NAFLD patients were diagnosed with ultrasonography (USG). A liver biopsy was not performed since it is an invasive method. In the control group, children with similar age and gender characteristics and followed in the Pediatric Gastroenterology outpatient clinic were included in the study. In both NAFLD and control groups, children with BMI > 25 kg/m2 were admitted. According to clinical and laboratory findings, USG and shear wave elastography (SWE) USG results, children were assigned to NAFLD and the control groups. ALT value > 40 IU/L is considered high (12). Serum samples for liver function tests, lipid profile and ferritin, B12, and vitamin D levels were obtained after 12 hours of fasting. BMI values of NAFLD patients and control group were calculated. BMI was calculated using the standard body weight (kg)/height2 (m2) formula.

Abdominal USG was performed after 6 hours of fasting. All patients were analyzed with special age-appropriate probes along with SWE. It was accepted as a reliable measurement if at least 5 valid measurements were taken by SWE.

The exclusion criteria of the control and NAFLD groups are underlying chronic liver disease, drug-induced liver damage, hematological diseases, hypothyroidism, hyperthyroidism, type 1 Diabetes Mellitus, adrenal insufficiency, renal failure, and thrombosis history. Furthermore, patients who took vitamin and iron preparations in the last year were also excluded from the study.

Statistical Analysis

IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for statistical analysis. While evaluating the study data, the normality assumption was checked with the Kolmogorov-Smirnov (K-S) test. In addition to descriptive statistical methods (mean, standard deviation, frequency), Student's t-test was applied for intergroup comparisons of normally distributed parameters between two groups, and the Mann-Whitney U test was deployed for comparisons of abnormal distributed variables. The data followed an abnormal distribution. Fisher-Freeman-Halton test and Continuity (Yates) Correction were made to compare qualitative data. Pearson correlation analysis was used to analyze the relationships between normally distributed variables, and Spearman's rho correlation analysis was utilized to examine the relationships between the abnormal parameters. A p value of less than 0.05 was considered significant.

RESULTS

There were 60 children in the NAFLD group and 66 children in the control group, respectively.. NAFLD and control groups were similar in age and gender (p=0.07; p=0.216). There was no difference in terms of BMI and waist circumference (p=0.06). The ALT (alanine aminotransferase) and GGT (gamma-glutamyl transferase) values of the patients in the NAFLD group were higher (p=0.01; p=0.03). There was no difference between the two groups in terms of AST (aspartate aminotransferase) and lipid profile. The intergroup comparison of total cholesterol, triglyceride, LDL and HDL values revealed that only HDL value was lower in the NAFLD group (p=0.02). In terms of HOMA-IR, an indicator of insulin resistance, NAFLD group had a higher score (p=0.020). Similarly, insulin levels were also higher in the NAFLD group while no difference was detected in blood sugar levels (p=0.001; p=0.31) (**Table 1**).

NAFLD and control groups were analyzed in terms of USG and SWE results. Grade 1 adiposity was observed in 35% (n=21) of the patients in the NAFLD group, grade 2 adiposity was observed in 55% (n=33) and grade 3 adiposity was observed in 10% (n=6). Liver sizes are larger in the NAFLD group due to adiposity. SWE assessment was higher in the NAFLD group (p=0.002) (**Table 2**).

Table 1. Comparison of general clinical findings between NAFLD and control groups

	NAFLD group mean±SD	Control group mean±SD	p
Female	34 (56.60%)	48 (73%)	[2]0.216
Male	26 (43.40%)	18 (27%)	
Age	13.10±2.70	12.20±3.80	[1]0.070
BMI (kg/m2)	28.60±2.80	27.40±2.50	[1]0.060
Waist circumference (cm)	94.50±13.00	92.40±10.90	[1]0.060
ALT (IU/L)	63.50±26.00 (54.50)	27.60±8.30 (16)	[3]0.010*
AST (IU/L)	45.90±37.70 (30)	28.30±11.00 (26.50)	[3]0.138
GGT (IU/L)	32.00±20.30 (24)	17.60±9.00 (12)	[3]0.030*
Total cholesterol (mg/dl)	178.50±38.00	162.50±36.50	[1]0.376
Trigliserid (mg/dl)	158.60±76.10 (120.50)	139.00±48.10 (147)	[3]0.247
HDL (mg/dl)	43.50±8.60	49.80±9.70	[1]0.020*
LDL (mg/dl)	104±39.60 (104.50)	104.10±27.60 (102)	[2]0.850
Homa-IR	4±2,50 (3.40)	2.30±0.60 (2.40)	[1]0.020*
Insulin (median)	20±11.30 (14.40)	12±3.70 (11)	[2]0.001*
Blood sugar (mg/dl)	92.80±8.00	90.10±10.50	[1]0.310

ALT, alanine aminotransferease; AST, serum aspartate aminotransferease; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease ; HOMA-IR, homeostatic model assessment insulin resistance ; 1Student t test ; 2Continuity (yates) correction ; 3Mann Whitney U test; *p<0,05

Table 2. Comparison of shear wave elastography (SWE) and USG results between NAFLD and control groups

	NAFLD group Mean±SD	Control group Mean±SD	p
Liver long axis (mm)	151.13±12.85	123.70±16.10	[1]0.001*
Liver SWE mean (m/s)	1.82±0.40 (1.74)	1.65±0.16 (1.60)	[2]0.002*
Hepatosteatosis n(%)			[3]0.001*
None	0 (0%)	66 (100%)	
Grade 1	21 (35%)	0 (0%)	
Grade 2	33 (55%)	0 (0%)	
Grade 3	6 (10%)	0 (0%)	

2Mann Whitney U test; 3Fisher Freeman Halton test; *p<0,05

Patients in the NAFLD group were compared with the control group in terms of micronutrients. While the ferritin level of the patients in the NAFLD group was higher (p=0.001); the B12 level was lower than the control group (p=0.006). In terms of vitamin D, there was no difference between the two groups (p=0.368) (**Table 3**).

Table 3. Evaluation of ferritin, B12 and vitamin D parameters between groups

	NAFLD group Mean±SD (median)	Control group Mean±SD (median)	p
Ferritin (mg / dl)	43.14±50.73 (26.80)	19.66±16.85 (16.80)	0.001*
B12 (mg / dl)	226.05±84.24 (215)	296.91±121.86 (269)	0.006*
Vitamin D (IU)	21.26±12.35 (18.80)	22.27±10.39 (22.50)	0.368

Mann Whitney U test; *p<0,05

DISCUSSION

NAFLD and related diseases, including insulin resistance and Diabetes Mellitus, may remain asymptomatic until they develop. NAFLD is the cause of nearly 7-11% of elevated liver function tests in obese patients. Moreover, in liver biopsies performed in obese patients, 74% of patients are diagnosed with NAFLD (13). Various clinical and biochemical indicators have been investigated for recognizing the presence of NAFLD in the early stage and distinguishing it from simple steatosis. These include clinical, biochemical, and metabolic laboratory results (14). In this study, NAFLD and control groups were evaluated in terms of age, gender, BMI, and waist circumference. The biggest risk factors for pediatric NAFLD are overweight and obesity. NAFLD is observed in 2-7% of normal-weight children as opposed to a rate of 50-80% in overweight and obese children (15). Manco et al. (16) reported that 92% of pediatric NAFLD patients had a Body Mass Index (BMI) higher than the 85th percentile and 84% of patients had a waist circumference greater than the 90th percentile. Furthermore, in a cross-sectional study, a significant correlation has been reported between waist circumference, adiposity, and the incidence of NAFLD with intra-abdominal fat tissue (17). Therefore, waist circumference may be a simple, useful, and beneficial screening tool in pediatric NAFLD.

AST and ALT, which are among the aminotransferases, are elevated in many liver diseases including NAFLD. In a multicenter study, AST and GGT were predictive for both NAFLD and NASH. However, it is not sufficient on its own to distinguish cases of NASH from simple steatosis thoroughly (18). It is also known that elevated aminotransferase levels are not specific for demonstrating liver damage and inflammation. In another pediatric study on NAFLD, approximately 65% of children with NASH had normal serum ALT and AST levels despite the progressed illness (19). Normal AST and ALT levels may not exclude liver injury and fibrosis in pediatric NAFLD. However, along with the high results in these tests, necessary screening tests for NAFLD should be performed in children who are overweight or obese. In the study, ALT values of in the NAFLD group were higher. In terms of AST, there was no difference between the two groups.

Serum lipid analysis results may show abnormal lipid metabolism (20). However, there is insufficient research on pediatric liver disease. In an adult study, analysis of molecular lipid concentrations in blood samples from 679 patients indicated that low-carbon number and double-bond triglycerols rose as lysophosphatidylcholine decreased in NAFLD patients (21). In present study, however, there was no difference in the lipid profile of NAFLD and control groups. It is expected that the HDL level is lower in the NAFLD group than in the control group.

Insulin Resistance (IR) is a significant metabolic abnormality related with NAFLD. It is also an important sign of disease seriousness in children. The intensity of IR was linked with hepatic fat deposition independent of general body adiposity. The prevalence of NAFLD is, therefore, higher in patients with hyperglycemia and Diabetes Mellitus. This mechanism has also been attributed by researchers to the fact that insulin resistance and hyperglycemia directly or indirectly increase advanced glycation products and proinflammatory cytokines and stimulate fibrosis (22). Similarly, insulin levels were higher in the NAFLD group in this study. Furthermore, there was no difference in bloodglucose levels.

USG is accepted as an effective method in diagnosing fatty liver in children and it has led to a great increase in NAFLD diagnosis lately. In NAFLD, the liver is usually expanded and it appears echogenic. This indicates the accumulation of fat in the parenchyma. However, it cannot determine the true extent of steatosis. The sensitivity of USG is significantly reduced in severely obese (BMI >40) and severely NASH individuals when hepatic fat deposition remains below 30% (23). USG can not reliably distinguish between simple steatosis and steatohepatitis and cannot exclude fibrosis. SWE can identify hepatic fibrosis in pediatric NAFLD using a technique comparable to ultrasound to noninvasively measure hepatic "stiffness" (24). In this study, NAFLD and the control group were analyzed for USG and SWE results. 35% of the patients in the NAFLD group had grade 1 adiposity, 55% had grade 2 adiposity, and 10% had grade 3 adiposity. It was also determined that liver sizes increased in the NAFLD group due to adiposity. The SWE assessment was higher in the NAFLD group, which was associated with fatty liver and fibrosis.

NAFLD is also related with aberrations in iron metabolism, and in the absence of genetic hemochromatosis, it causes elevations of intrahepatic free iron with slightly elevated serum ferritin and transferrin (25). This change is due to pro-inflammatory cytokines and adipokines.. In present study, ferritin levels in the NAFLD group were higher.

Vitamin B12 deficiency in the maternal diet has been associated with fatty liver in animal experiments (26). Defects in pathways related to fatty acid metabolism, amino acid metabolism, and glycolysis have also been observed in children born to mothers with B12 deficiency (27). Similarly, in this study, vitamin B12 levels in NAFLD group were lower than in the control.

Vitamin D deficiency is more common in obese patients than in normal-weight patients (28). Recent studies also indicate that low serum vitamin D is linked with insulin resistance and Type II diabetes, and that vitamin D supplementation can reduce insulin resistance (29). It has been noted that vitamin D deficiency in adults is related with liver steatosis, necroinflammation, and fibrosis in NAFLD patients (30). Contrary to these findings, there was no difference in vitamin D levels between NAFLD and control groups in this study. There is insufficient evidence regarding both the vitamin D level and the prevalence and severity of NAFLD as well as the effect of vitamin D supplementation in patients with NAFLD (31) Nevertheless, screening for vitamin D deficiency may still be beneficial for patients that are considered to be at high NAFLD risk.

Limitations of the Study

This study is subject to certain limitations. Patients in the NAFLD group were divided into three main groups based on the level of fatty liver. Due to the small number of patients in this group, the relationship between the level of fatty liver and ferritin, B12, and vitamin D levels could not be compared. Moreover, since liver biopsy could not be performed (since it is an invasive method and not an adequate indication), the patients were divided into two groups as NASH and simple steatosis. Therefore, an evaluation in terms of clinical findings and serum micronutrients was not possible.

CONCLUSION

Abdominal ultrasound and liver function tests are the most preferred tools for the diagnosis and screening of NAFLD in children. SWE can assist in the evaluation of hepatic fibrosis in a noninvasive manner compared to biopsy. All obese patients and children with a BMI >25 kg/m² are at risk and should be investigated for NAFLD. Serum ferritin, B12, and vitamin D levels of children on follow-up for NAFLD should be analyzed with consideration to their relationship with fatty liver.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gaziosmanpaşa Training and Research Hospital Ethics Committee (Date: 19.08.2020, Decision No: 144).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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