Zeynep Kamil Med J 2023;54(4):179–183
DOI: 10.14744/zkmj.2023.54926

ORIGINAL ARTICLE

Assessment of the relationship between non-alcoholic fatty liver disease and serum zinc levels in obese children and adolescents

[1]Eren ER
[2]Didem GÜLCÜ TAŞKIN

[1]Department of Pediatric Endocrinology, Tepecik Training and Research Hospital, İzmir, Türkiye
[2]Department of Pediatric Gastroenterology, Adana State Training and Research Hospital, Adana, Türkiye

ORCID ID
EE : 0000-0003-4770-0365
DGT : 0000-0002-2746-3799

ABSTRACT

Objective: As obesity rates increase, the prevalence of non-alcoholic fatty liver disease (NAFLD) increases as well. Numerous micronutrient deficiencies are associated with NAFLD. The development of NAFLD is thought to be influenced by zinc (Zn) deficiency. This study aims to investigate the relationship between NAFLD and serum Zn levels as well as some other parameters in obese children.

Material and Methods: This study examined the medical records of children and adolescents with obesity in the pediatric gastroenterology polyclinic 1 at the Adana City Training and Research Hospital between October 01, 2017 and August 31, 2022. Participants were evaluated in two groups, group 1 (NAFLD patients) and group 2 (non-NAFLD patients). Anthropometric and laboratory data were obtained from the patient's file.

Results: There were 91 patients (32 [35.1%] boys and 59 [64.8%] girls) who were 11.67±3.42 years old. Forty-two (46.1%) of the patients were affected by NAFLD, whereas 49 (53.8%) had normal results. The mean serum Zn level of the patients was 84.36±20.02 µg/dL. A statistically significant increase in serum Zn levels in group 2 compared to group 1 (89.96±7.99 µg/dL, 80.48±24.58 µg/dL; p=0.030) was observed. Other parameters did not show a significant difference between the groups (p>0.05). In logistic regression analysis, there was a significant negative correlation between NAFLD the presence and serum Zn level (p=0.049), but there was no significant correlation between the other parameters (p>0.05).

Conclusion: The present study is the first to examine the relationship between NAFLD and serum Zn in obese children in the literature. There was a statistically significant negative correlation between the presence of NAFLD and the level of serum Zn. According to these findings, Zn supplementation may be one of the treatment options for NAFLD management, and further studies with more patients are needed to investigate this possibility.

Keywords: Children, non-alcoholic fatty liver, obesity, zinc.

Cite this article as: Er E, Gülcü Taşkın D. Assessment of the relationship between non-alcoholic fatty liver disease and serum zinc levels in obese children and adolescents. Zeynep Kamil Med J 2023;54(4):179–183.

Received: February 24, 2023 **Revised:** March 14, 2023 **Accepted:** March 22, 2023 **Online:** October 06, 2023
Correspondence: Eren ER, MD. Tepecik Eğitim ve Araştırma Hastanesi, Çocuk Endokrinolojisi Kliniği, İzmir, Türkiye.
Tel: +90 232 469 69 69 **e-mail:** drerener1984@gmail.com
Zeynep Kamil Medical Journal published by Kare Publishing. Zeynep Kamil Tıp Dergisi, Kare Yayıncılık tarafından basılmıştır.

INTRODUCTION

It is important to note that non-alcoholic fatty liver disease (NAFLD) is a widespread, silent, chronic liver disease that may be mild or severe and without any obvious clinical symptoms.[1] Triglyceride (TG) content in hepatocytes >5% of liver weight results in liver steatosis.[2] When hepatic fatty acid intake, lipogenesis, lipid oxidation, and very low-density lipoprotein particles are disrupted, there is an excessive accumulation of TG in the hepatocytes and the consequent accumulation of liver fat.[3] NAFLD is a primary finding of obesity and metabolic syndrome in the liver.

Although NAFLD is seen in approximately 70% of obese patients, it is estimated to be around 35% in non-obese patients.[4,5] The accumulation of excess fat and insulin resistance are both associated with metabolic disorders such as TG, free-fatty acids, total cholesterol, low-density lipoprotein (LDL) cholesterol, and fasting blood sugar imbalances.[6,7] Recent evidence suggests that insulin resistance may contribute to NAFLD development.[7] In addition, disorders in the lipid profile also contribute to fatty liver disease.[8]

Zinc (Zn) deficiency has been reported in many studies.[9,10] This may result from low Zn intake, impaired liver hemostasis, and secondary effects of NAFLD, such as oxidative stress and inflammation.[11,12] Endoplasmic reticulum (ER) stress and fatty liver can be exacerbated in the presence of Zn deficiency. Studies have shown that Zn and Zn transporters reduce ER stress.[13,14] Furthermore, Zn regulates blood glucose homeostasis by affecting insulin secretion, receptor activation, and signal transduction.[15] As a result, Zn deficiency may worsen insulin resistance and diabetes.[16]

This study investigates the relationship between NAFLD and various blood parameters, especially Zn levels, in obese children.

MATERIAL AND METHODS

Patients

During October 01, 2017–August 31, 2022, children with obesity treated at Adana City Training and Research Hospital Pediatric Gastroenterology Polyclinic 1 were retrospectively analyzed. We included children from the age of 2 to 18 years who were evaluated as obese in our outpatient clinic (body mass index standard deviations [SDS] ≥2 SDS) based on the criteria of the World Health Organization.[17] The study excluded participants with chronic diseases (hormonal disorders, cancer, diabetes, cardiovascular diseases, hepatic or renal dysfunction, and malabsorption), using supplements (Zn, omega 3, or other supplements), or smoking habits. In accordance with the criteria for inclusion and exclusion, 91 patients (32 boys and 59 girls) with a mean age of 11.67±3.42 years were included in this study. An ultrasonography (USG) was performed to diagnose NAFLD following the American Society of Gastroenterology's standard criteria.[18] A total of two groups of patients were examined, one with NAFLD (group 1) and one without NAFLD (group 2). Ethical permission for the study was granted by Adana City Training and Research Hospital (dated December 29, 2022; decision number: 2348). All procedures followed the ethical standards specified in the Declaration of Helsinki. Parents of all patients were informed about the study's objectives and that their medical data could be published, and a consent form was obtained from their parents.

Table 1: Clinical characteristics of the patients

Features	Values
Number of patients	91
Gender (boy/girl)	32/59
NAFLD (yes/no)	42/49
Age (years)	11.67±3.42
Weight SDS	3.09±1.08
Height SDS	0.67±1.19
BMI SDS	2.81±0.65
Serum zinc level (µg/dL)	84.36±20.02
Serum 25(OH)D level (µg/dL)	22.84±7.33
Fasting blood glucose (mg/dL)	85.20±8.73
Insulin (U/L)	19,20±15,12
HOMA-IR	4.12±3.53
Total cholesterol (mg/dL)	169.63±44.34
Triglyceride (mg/dL)	117.32±55.36
LDL (mg/dL)	108.29±27.66
HDL (mg/dL)	44.77±9.01
AST (U/L)	25.52±7.99
ALT (U/L)	26.70±17.58
Serum TSH (mIU/L)	2.60±1.21
Serum ft4 (ng/dL)	0.87±0.11
Serum ft3 (ng/L)	4.11±0.52

NAFLD: Non-alcoholic fatty liver disease; SDS: Standard deviation scores; BMI: Body mass index; 25(OH)D: 25-hydroxyvitamin D; HOMA-IR: Homeostatic model assessment of insulin resistance; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TSH: Thyroid-stimulating hormone; ft4: Free thyroxine; ft3: Free triiodothyronine.

Analyses of Laboratory Data

Analyses were conducted on aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, LDL, cholesterol, triglyceride, and 25-hydroxyvitamin D (25[OH]D). Routine biochemical tests, including total cholesterol, TG, glucose, high-density lipoprotein, LDL cholesterol, ALT, and AST were performed using the ADVIA 1650 analyzer (Bayer, Pittsburgh, PA, USA). Serum 25(OH)D levels were measured using a radioimmunoassay method (DiaSorin; Stillwater, MN, USA) and a counter (1470 Wizard; Perkin-Elmer, Turku, Finland). Inductively coupled plasma mass spectrometry was employed to determine the Zn level (PerkinElmer, MA, USA, Zn normal range 70/120 µg/dL). Homeostatic model assessment of insulin resistance (HOMA-IR) was used to determine insulin resistance.[19] Detection of serum thyroid stimulating hormone (TSH), free thyroxine (fT4) and triiodothyronine (fT3), immunoenzymatic assays (TSH, fT4, and fT3), with commercial kits (Dimension EXL integrated chemistry system LOCI Module Siemens, Erlangen, Germany, TSH normal range 0.79/5.85 mIU/L, fT4 normal range 0.64/1.71 ng/dL, and fT3 normal range 2.6/6.5 ng/L).

Table 2: Comparison of the characteristics of the group with NAFLD and the group without NAFLD

Variables	Group with NAFLD (n=52)	Without NAFLD (n=79)	p
Age (years)	12.43±2.99	11.22±3.60	0.072
BMI SDS	2.96±0.63	2.72±0.66	0.080
Serum zinc level (µg/dL)	80.48±24.58	89.96±7.99	**0.030**
Serum 25(OH)D level (µg/dL)	21.83±7.42	23.60±7.26	0.320
Fasting blood glucose (mg/dL)	84.26±8.87	85.79±8.65	0.376
Insulin (U/L)	20.13±11.70	18.61±17.00	0.613
HOMA-IR	4.28±2.69	4.02±3.99	0.717
Total cholesterol (mg/dL)	168.75±56.48	170.05±38.18	0.915
Triglyceride (mg/dL)	110.62±50.15	121.65±58.45	0.317
LDL (mg/dL)	105.40±28.57	110.07±27.16	0.403
HDL (mg/dL)	44.71±8.67	44.81±9.26	0.954
AST (U/L)	25.10±7.66	25.79±8.24	0.663
ALT (U/L)	28.69±14.85	25.45±19.10	0.351
Serum TSH (mIU/L)	2.74±1.40	3.43±2.90	0.194
Serum ft4 (ng/dL)	0.85±0.12	0.88±0.14	0.211
Serum ft3 (ng/L)	3.99±0.48	4.11±0.78	0.496

NAFLD: Non-alcoholic fatty liver disease; SDS: Standard deviation scores; BMI: Body mass index; 25(OH)D: 25-hydroxyvitamin D; HOMA-IR: Homeostatic model assessment of insulin resistance; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TSH: Thyroid-stimulating hormone; ft4: Free thyroxine; ft3: Free triiodothyronine.

Statistical Analysis

SPSS 25 was employed for all statistical analyses (IBM Corp. released 2017. IBM SPSS statistics for Windows, version 25.0. Armonk, NY: IBM Corp.). Kurtosis–skewness tests were performed to determine whether the variables had normal distributions. A mean and SD or median (minimum-maximum) were presented for the results. Variables with a normally distributed distribution were given as mean±SD. A Chi-square test was used to analyze categorical variables between groups, and a Mann–Whitney U test was conducted to analyze non-categorical variables. The correlation analysis was conducted using Spearman and Pearson correlation coefficients. The relationship between variables was examined using a multivariate logistic regression model. Two-sided $p<0.05$ was accepted for statistical significance.

RESULTS

Ninety-one children (32 [35.1%] boys and 59 [64.8%] girls) aged 11.67±3.42 years participated in the study. The clinical characteristics of all participants are summarized in Table 1. HOMA-IR level was found to be >2.5 in 52 (57%) of the cases. NAFLD was found at least stage 1 and above in 42 (46.1%) patients, whereas this finding was not present in 49 (53.8%) patients. The USG image revealed grade 2 adiposity in 13 (14.2%) and grade 1 adiposity in 29 (70.1%) cases with NAFLD. There were 15 patients (16.4%) with elevated liver function tests. In this study, the mean serum Zn level was 84.36±20.02 µg/dL, and serum Zn levels of all individuals were within the normal range. The mean serum 25(OH)D level was 22.84±7.33 µg/dL.

In two groups of patients, NAFLD patients (group 1) and patients without NAFLD patients (group 2), the mean serum Zn levels of group 2 were significantly higher than those of group 1, and this difference was statistically significant (89.96±7.99 µg/dL vs. 80.48±24.58 µg/dL; $p=0.030$). In terms of other parameters, there were no significant differences between the groups ($p>0.05$) (Table 2).

Multivariate logistic regression analysis was performed to assess the relationship between some clinical parameters and NAFLD. While a significant negative correlation was observed between the presence of NAFLD and serum Zn level for the unadjusted model ($p=0.049$), other parameters did not show a significant correlation ($p>0.05$).

DISCUSSION

In this study, the variables related to the fatty liver were investigated, and it was demonstrated that there was a weak but significant negative correlation between serum Zn levels and NAFLD in obese children. This may be due to the limited number of participants. No significant correlation was found between serum lipids, insulin resistance, or BMI, and the presence of fatty liver. The present study is the first to examine the relationship between serum Zn levels and NAFLD in childhood.

Zn, a trace element in the structure of metabolic, anti-inflammatory, and antioxidant enzymes, may have impaired metabolism in chronic liver disease.[9] Studies in the literature have shown that patients with chronic active hepatitis, cirrhosis, and liver cancer have significantly reduced serum Zn levels.[20] Patients with chronic hepatitis C have been found to have a Zn deficiency, and Zn levels have improved with the administration of interferon.[21,22] Therefore, Zn supplementation improved liver function and long-term outcomes, including lower cumulative incidences of liver cancer in patients with chronic hepatitis C and liver cirrhosis.[23] Further, in individuals with chronic viral hepatitis, Zn levels are significantly associated with disease severity and prognosis. The presence of Zn deficiency or altered metabolism is also observed in individuals suffering from alcoholic liver disease.[24] In previous studies, Zn has been studied concerning viral hepatitis and alcoholic liver disease. There are limited data regarding the relationship between Zn level and NAFLD and previous studies have been conducted in adult patients.

Due to the increasing prevalence of obesity worldwide, the presence of NAFLD is an important risk factor for developing cirrhosis and liver cancer.[25] In addition, studies indicate that serum Zn concentrations are significantly lower in patients with NAFLD than in controls.[26] It has also been reported that lower Zn levels are related to higher hepatic fibrosis stages in patients with biopsy-proven NAFLD.[27] In a cross-sectional study of 300 subjects with NAFLD, it was found that defined by the NAFLD liver fat score,[28] significant hepatic fibrosis predicted by the fibrosis-4 index[29] is correlated with reduced levels of Zn.[30] In contrast to the results of these studies, a study that evaluated patients with NAFLD who underwent bariatric surgery did not find a significant relationship between serum Zn levels and the severity of hepatic steatosis, steatohepatitis, and fibrosis.[31] The results of animal studies indicate that Zn supplementation reduces the severity of liver steatosis in the periportal areas of the liver, reduces the accumulation of lipids in hepatocytes, improves glucose metabolism and insulin signaling, and reduces liver damage.[32–34] Several conditions are associated with Zn deficiency in patients with NAFLD, including oxidative stress and inflammation, insulin resistance, diabetes mellitus, obesity, hypertension, dyslipidemia, decreased Zn absorption from the gastrointestinal tract, and inadequate dietary Zn intake.[35] Our study examined the relationship between NAFLD and various factors that might affect the pathophysiology in obese children. Only the serum Zn level was significantly associated with the pathophysiology.

The limitations of our study were that it was retrospective, the single-center had a limited number of patients, the population under investigation was limited to children and adolescents with obesity, and there was no healthy/non-obese control group. In addition, other elements, such as selenium, that may play a role in the pathogenesis of NAFLD have not been evaluated.

CONCLUSION

The relationship between NAFLD and serum Zn in children and adolescents with obesity has been demonstrated for the first time in the present study. Patients with obesity showed a weak, but statistically significant negative correlation between NAFLD and serum Zn. This study suggests that Zn supplementation may be an effective treatment option for NAFLD. A prospective, randomized, controlled study with a more significant number of participants is required in this area to identify the relationship between Zn treatment and NAFLD.

Statement

Ethics Committee Approval: The Adana State Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 29.12.2022, number: 2348).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – EE, DGT; Design – EE; Supervision – EE; Resource – EE; Materials – EE, DGT; Data Collection and/or Processing – DGT; Analysis and/or Interpretation – EE; Literature Search – EE, DGT; Writing – EE; Critical Reviews – EE.

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

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

REFERENCES

1. Sorrentino P, Tarantino G, Conca P, Perrella A, Terracciano ML, Vecchione R, et al. Silent non-alcoholic fatty liver disease—a clinical–histological study. J Hepatol 2004;41:751–7.
2. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.
3. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. J Gastroenterol 2013;48:434–41.
4. Farrell GC, George J, Hall de la MP, McCullough AJ, editors. Fatty Liver Disease: NASH and Related Disorders. 1st ed. New Jersey: Wiley-Blackwell; 2004.
5. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: From steatosis to cirrhosis. Hepatology 2006;43:S99–112.
6. Byrne CD, Targher G. NAFLD: A multisystem disease. J Hepatol 2015;62:S47–64.
7. Lambert JE, Ramos–Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology 2014;146:726–35.
8. Lucero D, Zago V, López GI, Graffigna M, López GH, Fainboim H, et al. Does non-alcoholic fatty liver impair alterations of plasma lipoproteins and associated factors in metabolic syndrome? Clin Chim Acta 2011;412:587–92.
9. Himoto T, Masaki T. Associations between zinc deficiency and metabolic abnormalities in patients with chronic liver disease. Nutrients 2018;10:88.
10. Kosari F, Jamali R, Ramim T, Mosavi Jahan Abad E. The correlation between serum zinc level and liver histology in non-alcoholic steatohepatitis. Iran J Pathol 2019;14:17–25.
11. Vranešić Bender D, Nutrizio M, Jošić M, Ljubas Kelečić D, Karas I, Premužić M, et al. Nutritional Status and nutrition quality in patients with non-alcoholic fatty liver disease. Acta Clin Croat 2017;56:625–34.
12. Prasad AS. The role of zinc in gastrointestinal and liver disease. Clin Gastroenterol 1983;12:713–41.
13. Kim MH, Aydemir TB, Kim J, Cousins RJ. Hepatic ZIP14-mediated zinc transport is required for adaptation to endoplasmic reticulum stress. Proc Natl Acad Sci U S A 2017;114:E5805–14.
14. Sakurai H, Adachi Y. The pharmacology of the insulinomimetic effect of zinc complexes. BioMetals 2005;18:319–23.

15. Bjørklund G, Dadar M, Pivina L, Doşa MD, Semenova Y, Aaseth J. The role of zinc and copper in insulin resistance and diabetes mellitus. Curr Med Chem 2020;27:6643–57.
16. Himoto T, Yoneyama H, Deguch A, Kurokohchi K, Inukai M, Masugata H, et al. Insulin resistance derived from zinc deficiency in non-diabetic patients with chronic hepatitis C. Exp Ther Med 2010;1:707–11.
17. World Health Organization. Obesity and overweight. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed Dec 24, 2022.
18. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705–25.
19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
20. Pramoolsinsap C, Promvanit N, Komindr S, Lerdverasirikul P, Srianujata S. Serum trace metals in chronic viral hepatitis and hepatocellular carcinoma in Thailand. J Gastroenterol 1994;29:610–5.
21. Takagi H, Nagamine T, Abe T, Takayama H, Sato K, Otsuka T, et al. Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. J Viral Hepat 2001;8:367–71.
22. Matsuoka S, Matsumura H, Nakamura H, Oshiro S, Arakawa Y, Hayashi J, et al. Zinc supplementation improves the outcome of chronic hepatitis C and liver cirrhosis. J Clin Biochem Nutr 2009;45:292–303.
23. Hiraoka A, Nagamatsu K, Izumoto H, Adachi T, Yoshino T, Tsuruta M, et al. Zinc deficiency as an independent prognostic factor for patients with early hepatocellular carcinoma due to hepatitis virus. Hepatol Res Off J Jpn Soc Hepatol 2020;50:92–100.
24. Kang YJ, Zhou Z. Zinc prevention and treatment of alcoholic liver disease. Mol Aspects Med 2005;26:391–404.
25. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: An emerging menace. J Hepatol 2012;56:1384–91.
26. Akdas S, Yazihan N. Serum zinc level and dietary zinc intake status in non-alcoholic fatty liver disease: A meta-analysis and systematic review. Hepatol Forum 2020;1:59–67.
27. Ito T, Ishigami M, Ishizu Y, Kuzuya T, Honda T, Ishikawa T, et al. Correlation of serum zinc levels with pathological and laboratory findings in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2020;32:748–53.
28. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009;137:865–72.
29. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317–25.
30. Kim MC, Lee JI, Kim JH, Kim HJ, Cho YK, Jeon WK, et al. Serum zinc level and hepatic fibrosis in patients with nonalcoholic fatty liver disease. PLoS One 2020;15:e0240195.
31. Bertol FS, Araujo B, Jorge BB, Rinaldi N, Carli LAD, Tovo CV. Role of micronutrients in staging of nonalcoholic fatty liver disease: A retrospective cross-sectional study. World J Gastrointest Surg 2020;12:269–76.
32. Gatiatulina ER, Sheina EA, Nemereshina ON, Popova EV, Polyakova VS, Agletdinov EF, et al. Effect of Zn supplementation on trace element status in rats with diet-induced non-alcoholic fatty liver disease. Biol Trace Elem Res 2020;197:202–12.
33. Qi Y, Zhang Z, Liu S, Aluo Z, Zhang L, Yu L, et al. Zinc supplementation alleviates lipid and glucose metabolic disorders induced by a high-fat diet. J Agric Food Chem 2020;68:5189–200.
34. Vivero A, Ruz M, Rivera M, Miranda K, Sacristán C, Espinosa A, et al. Zinc supplementation and strength exercise in rats with type 2 diabetes: Akt and PTP1B phosphorylation in nonalcoholic fatty liver. Biol Trace Elem Res 2021;199:2215–24.
35. Barbara M, Mindikoglu AL. The role of zinc in the prevention and treatment of nonalcoholic fatty liver disease. Metab Open 2021;11:100105.