



Prevalence and Predictors of Non-Alcoholic Fatty Liver Disease in Obese and Overweight Children in the Northwest of Iran

Shahsanam Gheibi¹, Farzad Maleki², Saeid Safiri^{3,4}, Hadi Esmaeili GouvarchinGhaleh⁵, Bahman Mansouri Motlagh⁶ and Marjan Hosseinpour^{1,*}

¹Maternal and Childhood Obesity Research Center, Urmia University of Medical Sciences, Urmia, Iran

²Social Determinants of Health Research Center, Urmia University of Medical Sciences, Urmia, Iran

³Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran

⁴Department of Community Medicine, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

⁵Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

⁶Department of Pathobiology, Faculty Veterinary Medicine, Urmia University, Urmia, Iran

*Corresponding author: Maternal and Childhood Obesity Research Center, Urmia University of Medical Sciences, Urmia, Iran. Tel: +98-4432237077- Ext: 543, Email: husseinpourm@yahoo.com

Received 2019 April 12; Revised 2019 August 07; Accepted 2019 September 19.

Abstract

Background: Over recent decades, with the increase in the prevalence of childhood overweight/obesity, the prevalence of pediatric non-alcoholic fatty liver disease (NAFLD) has increased.

Objectives: The aim of this study was to investigate the prevalence of NAFLD and its predisposing factors in overweight and obese children of Urmia, Northwest of Iran.

Methods: In this cross-sectional study, a total of 843 children aged 2 to 19 years were recruited out of 10800 children referred to the Digestive Disease Clinic of Shahid Motehari Hospital during 2016-2017. Anthropometric and laboratory measurements and abdominal ultrasound were performed for the children. Demographic data and their medical history were collected by a questionnaire. Unconditional logistic regression was used to predict the predisposing factors of NAFLD.

Results: Fatty liver was diagnosed by ultrasound in 9.5% of overweight and 21.4% of obese children. The prevalence of NAFLD in obese children was 9.26% for the 2-5.9-year age group, 22.3% for the 6-11.9-year age group and 35.5% for the 12-19-year age group. Compared to the normal liver group, the adjusted odds ratios (ORs) [95% confidence interval (CI)] for predictive factors of NAFLD were as follows: ALT: 1.05 (1.03-1.09), ALK: 1.02 (1.01-1.03), AST: 1.04 (1.02-1.08), triglycerides: 1.1 (1.08-1.21), TSH: 1.18 (1.1-1.40), FBS: 1.04 (1.01-1.08) and HOMA-IR: 1.19 (1.03-1.38). Compared to the age group of 2-5.9 years, the odds ratio of NAFLD was increased by 4 and 8 times in the age group 6-11.9 and 12-19 years, respectively.

Conclusions: There was a strong relationship between pediatric NAFLD and ALT, AST and HOMA-IR in the overweight and obese children. Our findings emphasized the importance of prevention of obesity and early intervention to prevent abnormalities among obese children.

Keywords: Non-Alcoholic Fatty Liver Disease, Pediatric Obesity, Overweight

1. Background

Non-alcoholic fatty liver disease (NAFLD) refers to a group of liver abnormalities from steatosis to steatohepatitis, which can lead to cirrhosis and liver failure (1, 2). Moreover, NAFLD causes several pediatric serious comorbidities and behavioral problems (3, 4). Over the past three decades, with a significant increase in the prevalence of childhood obesity and overweight, the prevalence of pediatric NAFLD has increased (5), but because of the lack of reliable noninvasive screening tools, the real prevalence of NAFLD in children remains uncertain (6, 7). The global prevalence of NAFLD in children is about 7.6 and approxi-

mately 34% in obese children. Asia has the highest NAFLD prevalence, worldwide (8). Almost 20% of Asian obese children and adults have NAFLD, and it is more prevalent in the Middle East (9). Moreover, the NAFLD prevalence is over 30% in Iranian population (10). The prevalence of NAFLD in Iranian children has not recently been reported. Since Iran is one of the top ten countries in Asia with a high prevalence of obesity, the NAFLD prevalence in Iran is expected to increase in the future (9). In addition, given the industrial development and economic growth as well as lifestyle changes in developing countries like Iran, the rate of obesity, especially in children is rising (11). The prevalence of

childhood obesity/overweight in the northwest of Iran is higher than that of its southern and central regions (12). Therefore, NAFLD is the most common liver abnormality in the first and second decades of life and its prevalence is increasing rapidly (13, 14).

2. Objectives

Accordingly, we conducted a large-scale study on pediatric NAFLD to evaluate its prevalence and predisposing factors in overweight and obese children aged 2 to 19 years in Urmia.

3. Methods

3.1. Study Population

In this cross-sectional study, we recruited 10800 children aged 2 - 19 years who were referred to the Digestive Disease Clinic of Shahid Motahari Hospital of Urmia University of Medical Science, between March 2016 and December 2017. Of the 10800 children, 843 samples (425 males) were eligible and agreed to participate in the study. A questionnaire was used for collecting the participants' demographic information, medical history and alcohol consumption. Anthropometric and laboratory measurements and abdominal ultrasound for liver echogenicity and size were performed for the children. Inclusion criteria were children aged 2 - 19 years, the body mass index (BMI) of higher than 85th percentile, the absence of markers of hepatitis B virus (HBV) and C virus (HCV) in serum, and Wilson's disease. The Ethics Committee of Urmia University of Medical Sciences approved our study protocol. Parents were informed about the study objectives and completed the written consent.

Transabdominal ultrasonography was performed by two trained and experienced radiologists using a Samsung ultrasound machine (Samsung Medison, Seoul, Korea) with a 5 to 7 MHz transducer probe for diagnosis of NAFLD. The ultrasound criteria for the diagnosis of NAFLD consist of Grade 1: a mild increase in liver echogenicity, Grade 2: a moderate increase in liver echogenicity and the limits of the diaphragm and intra-hepatic arteries are slightly faded, and Grade 3: a severe increase in liver echogenicity and the limits of the diaphragm and intra-hepatic arteries are severely faded and the posterior part of the right lobe is hardly observed. None of these symptoms was described as the normal liver (15).

3.2. Anthropometric Measurements

BMI was calculated as weight (kg)/height (m²). Z-scores of weight (Z-weight), height (Z-height) and BMI (Z-BMI) were calculated based on the World Health Organization (WHO) reference data for gender and age.

3.3. Biochemical Analysis

To measure blood glucose levels and serum lipids, venous blood samples were collected after 8 - 10 hours of overnight fasting. The samples were subsequently analyzed at a certified laboratory. Levels of serum Alanineaminotransferase (ALT), Aspartateaminotransferase (AST), and alkaline phosphate (ALK) were measured using commercial kits (Pars Azmoon, Tehran, Iran). Triglycerides (TG), cholesterol (Chol), low density lipoprotein (LDL)-cholesterol and high density lipoprotein (HDL)-cholesterol were also measured by enzymatic kits (Pars Azmoon, Tehran, Iran). Thyroid stimulating hormone (TSH) was measured by IRMA (Immunotech, Czech Republic). HBV surface antigens and HCV antibodies were measured by the enzyme-linked immunosorbent assay (ELISA) (Acon kit). HBsAg was detected using Biorad kits and anti-HCV was detected by Biomerieux kits. Fasting blood sugar (FBS) was measured enzymatically by auto-analyzer (Hitachi, Tokyo, Japan). Serum fasting insulin was measured using a chemiluminescent immunoassay method (DiaSorin, Liaison, Italy). The cutoff of ≥ 110 mg/dL was used for high FBS, ≥ 140 mg/dL for high TG, ≥ 190 for high total Chol and ≥ 115 mg/dL for high LDL levels. The cutoff of 440 U/L for elevated ALT levels and ≥ 20 mU/mL for high fasting insulin were used. According to the homeostatic model assessment of insulin resistance (HOMA-IR) method, Insulin resistance was calculated through the following formula:

$$\text{Fasting glucose (mg/dL)} \times \text{fasting insulin (mU/L)} / 450.$$

3.4. Statistical Analysis

The quantitative variables were described as mean and 95% confidence intervals and qualitative variables were described using frequency (%). The normal distribution was examined by the Kolmogorov-Smirnov test. Student's *t*-test and one-way analysis of variance (ANOVA) were used to compare the means in NAFLD and normal liver groups and the chi-square test was applied for categorical variables. Unconditional logistic regression was used to estimate the odds ratios (ORs) [95% confidence interval (CI)] for predictive factors of NAFLD. For adjusting potential confounding variables, the stepwise backward multivariable logistic regression analysis was done. Significant predictors of NAFLD at univariable analysis, considering clinically oriented confounder strategy were evaluated in three distinct multivariable logistic regression models. Model 1 consisted of age, gender, HDL-cholesterol, Z-BMI, ALT, AST, triglycerides, glucose, insulin and ALK. Model 2 consisted of age, gender, HDL-cholesterol, Z-BMI, ALT, AST and ALK and model 3 included age, gender, Z-BMI, FBS, TG, TSH, ALT, AST, glucose, insulin and ALK. In addition to gender, all predictors were evaluated as continuous variables.

4. Results

4.1. Characteristics of the Study Population

Of the 843 participants, 506 subjects (59.8%) were overweight and 337 subjects (40.2%) were obese. The percentage of subjects in the 2 - 5.9-year age group, 6 - 11.9-year age group and 12 - 19-year age group was 39.74 %, 48.87% and 11.39%, respectively. Eleven percent of the 843 children (n = 93) had NAFLD. The prevalence of NAFLD in overweight and obese children was 9.5 and 21.4 %, respectively. The prevalence of NAFLD in obese children was 9.3% for the age group of 2 - 5.9 years, 22.3% for the age group of 6 - 11.9 years and 35.6% for the age group 12 - 19 years (Figure 1). Among children with NAFLD, 65 cases (69%) showed grade one and 28 cases (31%) showed grade two. Table 1 represents the measurements of the children with and without NAFLD.

4.2. Comparison of Children with and Without NAFLD

There was no statistically significant difference in the age of children with and without NAFLD ($P = 0.766$). There was no significant difference between females and males ($P = 0.491$). The levels of ALT ($P < 0.0001$), ALK ($P < 0.0001$), AST ($P < 0.0001$), triglycerides ($P < 0.0001$), cholesterol ($P < 0.0001$), TSH ($P = 0.003$), FBS ($P = 0.0092$) and HOMA-IR ($P = 0.022$) were higher in NAFLD children. No statistically significant difference was observed between two groups in terms of gender ($P = 0.49$), LDL-cholesterol ($P = 0.63$), insulin ($P = 0.223$), Z-weight ($P = 0.15$) and Z-BMI ($P = 0.33$).

Serum ALT was found higher than 30 U/L in 68 subjects (8%) and higher than 40 U/L in 35 subjects (25%), of whom 40 children in the former and 22 in the latter group had NAFLD ($P = 0.025$).

4.3. The Relation Between Biochemical Variables in Children with and Without NAFLD

In the adjusted logistic regression analysis, compared to the age group of 2 - 5.9 years, the odds of NAFLD was increased by 4 and 8 times in the age group of 6 - 11.9 and 12 - 19 years, respectively. An increase of 10 U/L in ALT, AST and ALK increased the odds of NAFLD 101%, 127% and 2%, respectively. An increase of 10 mg/dL in TG was associated with an 8% increase and also 10 μ lU/mL of TSH with a 55% increase in the odds of NAFLD. An increase of 10 mg/dL in glucose was associated with a 34% increase in the odds of NAFLD. An increase of one unit in HOMA-IR was associated with a 19% increase in the odds of NAFLD (Table 2).

5. Discussion

This study aimed to determine the prevalence of NAFLD in obese and overweight children and its association with biochemical parameters. Our results indicated

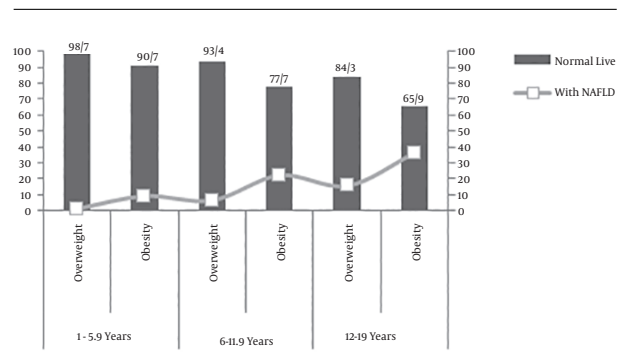


Figure 1. Prevalence of non-alcoholic fatty liver disease by age group

that the prevalence of NAFLD in overweight and obese children was 9.5% and 21.4 %, respectively. Alavian et al. (16) reported the NAFLD prevalence in Iranian obese and overweight children aged 7-18 years 31.3% and 11.1%, respectively. This percentage is associated with the population's characteristics as well as diagnostic techniques (17). In the present study and also Alavian study, ultrasonography was used as the diagnostic tool, however the rate of the age group of 12 - 19 years was less than that of younger children in our study. Forty percent of the children were younger than 6 years with a 3.8% prevalence of NAFLD, whereas 11.39% of the subjects were in the age group 12 - 19 years with an age-specific prevalence of 25%. More interestingly, the age-specific prevalence in overweight and obese children aged 12 - 19 years was 15.69 and 35.56, respectively. It should be noted that adolescent changes, such as hormonal changes in puberty, fat accumulation in the liver and more tendency to eat harmful foods may increase the prevalence of both obesity and NAFLD (18, 19). Various results have been reported in other studies, for example, in Germany, the NAFLD prevalence was 28% in obese children aged 8 - 19 years (20), in Brazil, it was 20.5% in obese children (21), in Turkey, a rate of 60.8% has reported in obese children aged 4 - 17 years (22) and in Mumbai, India its incidence has announced 12.9% in obese children aged 11 - 15 years (23).

In addition, in our study the prevalence of Grade 1, 2, and 3 fatty liver was 69, 31, and 0%, respectively. A similar research has been conducted in Iran reporting the 84.1% of mild, 14.3% of moderate and 1.6% of severe (16) grades of fatty liver. It should be noted that liver biopsy is the gold standard for assessing the severity of NAFLD (24). Ultrasonography may only predict mild grades. Recent studies have shown that children with slightly elevated ALT may have significant histological disturbances (25).

Our study indicated that the mean ALT, ALK and AST of children with NAFLD were significantly higher than the children without NAFLD. The increased levels of ALT and AST seem to be associated with NAFLD, clinically and histo-

Table 1. Comparison of the Demographic, Anthropometric and Biochemical Characteristics Between Non-Alcoholic Fatty Liver Disease and Normal Liver^a

Variables	Normal Liver (N = 750)	NAFLD (N = 93)	P Value
Gender (male/female, n)	375/375	50/43	0.49
Mean age	7.3 [7.05 - 7.54]	7.6 [7.36 - 7.83]	0.766
Z-weight (SDS)	1.39 [1.33 - 1.46]	1.54 [1.35 - 1.73]	0.15
Z-BMI (SDS)	1.59 [1.54 - 1.64]	1.66 [1.53 - 1.79]	0.33
FBS (mg/dL)	83.43 [82.81 - 84.04]	87.46 [85.84 - 89.07]	< 0.0001
TG (mg/dL)	103.3 [100.97 - 105.62]	131.26 [117.62 - 144.89]	< 0.0001
Chol (mg/dL)	128.32 [125.91 - 130.73]	159.6 [151.55 - 167.66]	< 0.0001
LDL (mg/dL)	107.59 [107.06 - 108.01]	107.09 [103.15 - 111.02]	0.63
HDL (mg/dL)	36.44 [36.07 - 36.81]	39.13 [37.6 - 40.66]	< 0.0001
TSH (μ Iu/mL)	2.24 [2.14 - 2.34]	2.73 [2.35 - 3.11]	0.003
Insulin (μ Iu/mL)	12.73 [12.38 - 13.08]	13.39 [12.33 - 14.46]	0.223
Cortisol (mcg/dL)	16.22 [15.85 - 16.6]	14.51 [13.18 - 15.83]	0.013
ALT (U/L)	18.64 [18.11 - 19.17]	35.54 [29.74 - 41.35]	< 0.0001
AST (U/L)	19.82 [19.41 - 20.23]	29.85 [26.2 - 33.49]	< 0.0001
ALK (U/L)	295.33 [283 - 307]	461.86 [398 - 524]	< 0.0001
HOMA-IR	2.6 [2.51 - 2.61]	3.01 [1.09 - 8.7]	0.022
Age groups			< 0.0001
2 - 5.9	322 (96.12)	13 (3.88)	
6 - 11.9	356 (86.41)	56 (13.59)	
12 - 19	72 (75.0)	24 (25)	

^aValues are expressed as mean [95% confidence intervals] or No. (%).

logically (26). These enzymes are commonly found in the liver cells and can enter the bloodstream due to liver damage, so the elevated levels of enzymes in the blood may be a marker for liver degeneration (27). Other studies verified the strong association between the elevated levels of liver enzymes and NAFLD (28, 29). Other studies also showed that ALT is associated with markers of oxidative stress and inflammation, which can lead to liver degeneration (30).

Interestingly, our study indicated that the NAFLD group had higher mean FBS and also they had high-insulin resistance, as estimated with an index of HOMA-IR. This is in agreement with the results of other studies (31, 32). Insulin prevents free fatty acid oxidation, and therefore, hyperinsulinemia may increase hepatotoxicity and steatosis by increasing free fatty acid in hepatocytes as well as generating free radical formation (33, 34).

Moreover, our results showed that TG might predict NAFLD in obese children. The obesity and accumulation and circulation of saturated fatty acids in the liver can result in liver degeneration by activating the apoptotic process (35). Free fatty acids are exposed to oxidative stress and by increasing β -oxidation can result in mitochondria degradation and the elevated levels of active oxygen is

possibly a major cause for development of NAFLD (36). The pathogenesis of NAFLD can be introduced by a model, which is a two-hit theory, in which it is stated that the disease is caused by the first-hit due to insulin resistance, obesity and dyslipidemia, and due to the second-hits, such as oxidative stress, pro-inflammatory cytokines and intestinal bacterial toxins, ultimately inflammation and fibrosis of liver cells are occurred (37). On the other hand, liver enzymes, like ALT, as an indicator of oxidative stress and inflammation are factors resulting in insulin resistance (38).

Our findings revealed that TSH might predict NAFLD in obese children. The level of TSH has a remarkable effect on metabolism. Patients with overt hypothyroidism have the elevated total cholesterol and LDL-cholesterol levels (39, 40). In addition, NAFLD and hypothyroidism exert similar significant effects on metabolism, such as decreasing the fatty acid beta-oxidation and increasing lipid peroxidation. These changes are the leading source of oxidative stress and cell injury in the liver tissue (41). Regarding thyroid function, various studies have confirmed that the level of TSH in children with NAFLD is significantly higher than that of the control group and it is associated with an increase in the grade of NAFLD (39, 42). Our study results

Table 2. Crude and Adjusted Odds Ratios (OR) and 95% Confidence Intervals (CI) for Non-Alcoholic Fatty Liver Disease in Various Models

Variables	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
Model 1		
Age, y		
2 - 5.9	1	1
6 - 11.9	3.90 (2.09 - 7.26)	4.89 (1.75 - 13.66)
12 - 19	8.26 (4.01 - 16.99)	9.56 (3.12 - 30.32)
Gender		
Male	1	1
female	0.86 (0.56 - 1.33)	1.11 (0.55 - 2.22)
Model 2		
Insulin (μ Iu/mL)	1.3 (0.85 - 1.97)	0.98 (0.91 - 1.04)
HOMA-IR	1.11 (1.02 - 1.25)	1.19 (1.03 - 1.38)
Glucose (mg/dL)	1.55 (1.24 - 1.92)	1.34 (1.02 - 1.75)
Z-BMI (SDS)	1.18 (0.85 - 1.65)	2.7 (1.84 - 3.96)
Model 3		
TG (mg/dL)	1.12 (1.08 - 1.19)	1.08 (1.03 - 1.13)
Chol (mg/dL)	1.22 (1.16 - 1.3)	1.16 (1.09 - 1.23)
LDL (mg/dL)	0.95 (0.76 - 1.18)	0.92 (0.76 - 1.01)
Low HDL cholesterol (mg/dL)	1.88 (1.39 - 2.46)	1.58 (1.11 - 2.22)
TSH (μ Iu/mL)	1.16 (1.66 - 14.3)	1.55 (0.98 - 2.43)
ALT (U/L)	2.22 (1.84 - 2.75)	2.01 (1.64 - 2.46)
AST (U/L)	2.34 (1.89 - 2.89)	2.27 (1.81 - 2.86)
ALK (U/L)	1.04 (1.03 - 1.06)	1.02 (1.01 - 1.03)

^aModel 1 adjusted for age, gender, HDL-Cholesterol, Z-BMI, ALT, AST, triglycerides, glucose, insulin, and ALK; Model 2 adjusted for age, gender, HDL-Cholesterol, Z-BMI, ALT, AST and ALK; Model 3 adjusted for age, gender, Z-BMI, TSH, ALT, AST, glucose, insulin, and ALK.

indicated that LDL-cholesterol can not predict NAFLD. Furthermore, we found that gender is not correlated with the prevalence of NAFLD.

To our knowledge, this is the first large-scale study on the prevalence of NAFLD and its predisposing factors in overweight and obese children of Urmia, Northwest of Iran. Despite our large sample size, we faced some limitations. First, its cross-sectional design cannot prove a causal relationship due to the absence of a normal-weight children group as well as the loss of patients for follow-up. Secondly, the gold standard for diagnosis is liver biopsy. The ultrasound cannot detect severe inflammation and fibrosis. However, we used ultrasound as a non-invasive, accessible and appropriate method for epidemiological studies with large sample size.

5.1. Conclusions

In conclusion, 21.4% of the obese children had NAFLD. Based on our findings, ALT, AST and HOMA-IR were associated with NAFLD and can predict the progression of the disease. Moreover, our findings indicated the importance of prevention of obesity and early intervention to prevent abnormalities to decrease morbidity among obese children. Further studies, perfectly executed with reference methods are needed for better determination of the status of obese children with NAFLD.

Acknowledgments

This study was funded by the Maternal and Childhood Research Center, Urmia University of Medical Science, Iran.

Footnotes

Authors' Contribution: Study concept and design: Shahsanam Gheibi, Marjan Hosseinpour, and Farzad Maleki; acquisition of data: Shahsanam Gheibi, Hadi Esmaeili GouvarchinGhaleh, and Bahman Mansouri Motlagh; analysis and interpretation of data: Farzad Maleki, Saeid Safiri, and Marjan Hosseinpour; drafting of the manuscript: Shahsanam Gheibi, Farzad Maleki, and Marjan Hosseinpour; critical revision of the manuscript for important intellectual content: Shahsanam Gheibi and Saeid Safiri; statistical analysis: Farzad Maleki and Marjan Hosseinpour; administrative, technical, and material support: Shahsanam Gheibi, Farzad Maleki, and Marjan Hosseinpour; study supervision: Shahsanam Gheibi and Marjan Hosseinpour.

Conflict of Interests: The authors disclose no conflicts of interests.

Ethical Approval: The Ethics Committee of Urmia University of Medical Sciences approved our study protocol.

Funding/Support: This study was funded by the Maternal and Childhood Research Center, Urmia University of Medical Science, Iran.

References

1. Brunt EM. Pathology of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol.* 2010;7(4):195-203. doi: [10.1038/nrgastro.2010.21](https://doi.org/10.1038/nrgastro.2010.21). [PubMed: [20195271](https://pubmed.ncbi.nlm.nih.gov/20195271/)].
2. Molleston JP, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol.* 2002;97(9):2460-2. doi: [10.1111/j.1572-0241.2002.06003.x](https://doi.org/10.1111/j.1572-0241.2002.06003.x). [PubMed: [12358273](https://pubmed.ncbi.nlm.nih.gov/12358273/)].
3. Mazzone L, Postorino V, De Peppo L, Della Corte C, Lofino G, Vassena L, et al. Paediatric non-alcoholic Fatty liver disease: Impact on patients and mothers' quality of life. *Hepat Mon.* 2013;13(3): e7871. doi: [10.5812/hepatmon.7871](https://doi.org/10.5812/hepatmon.7871). [PubMed: [23745129](https://pubmed.ncbi.nlm.nih.gov/23745129/)]. [PubMed Central: [PMC3669678](https://pubmed.ncbi.nlm.nih.gov/PMC3669678/)].

4. Malespin M, Slesman B, Lau A, Wong SS, Cotler SJ. Prevalence and correlates of suspected nonalcoholic fatty liver disease in Chinese American children. *J Clin Gastroenterol*. 2015;**49**(4):345-9. doi: [10.1097/MCG.0000000000000121](https://doi.org/10.1097/MCG.0000000000000121). [PubMed: [24667593](https://pubmed.ncbi.nlm.nih.gov/24667593/)].
5. Manco M, Bottazzo G, DeVito R, Marcellini M, Mingrone G, Nobili V. Nonalcoholic fatty liver disease in children. *J Am Coll Nutr*. 2008;**27**(6):667-76. doi: [10.1080/07315724.2008.10719744](https://doi.org/10.1080/07315724.2008.10719744). [PubMed: [1915426](https://pubmed.ncbi.nlm.nih.gov/1915426/)].
6. Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology*. 2013;**57**(4):1357-65. doi: [10.1002/hep.26156](https://doi.org/10.1002/hep.26156). [PubMed: [23175136](https://pubmed.ncbi.nlm.nih.gov/23175136/)]. [PubMed Central: [PMC3622816](https://pubmed.ncbi.nlm.nih.gov/PMC3622816/)].
7. Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. *J Hepatol*. 2013;**58**(5):1007-19. doi: [10.1016/j.jhep.2012.11.021](https://doi.org/10.1016/j.jhep.2012.11.021). [PubMed: [23183525](https://pubmed.ncbi.nlm.nih.gov/23183525/)].
8. 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**(10):e0140908. doi: [10.1371/journal.pone.0140908](https://doi.org/10.1371/journal.pone.0140908). [PubMed: [26512983](https://pubmed.ncbi.nlm.nih.gov/26512983/)]. [PubMed Central: [PMC4626023](https://pubmed.ncbi.nlm.nih.gov/PMC4626023/)].
9. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. *J Hepatol*. 2017;**67**(4):862-73. doi: [10.1016/j.jhep.2017.06.003](https://doi.org/10.1016/j.jhep.2017.06.003). [PubMed: [28642059](https://pubmed.ncbi.nlm.nih.gov/28642059/)].
10. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. 2018;**15**(1):11-20. doi: [10.1038/nrgastro.2017.109](https://doi.org/10.1038/nrgastro.2017.109). [PubMed: [28930295](https://pubmed.ncbi.nlm.nih.gov/28930295/)].
11. Gupta N, Goel K, Shah P, Misra A. Childhood obesity in developing countries: Epidemiology, determinants, and prevention. *Endocr Rev*. 2012;**33**(1):48-70. doi: [10.1210/er.2010-0028](https://doi.org/10.1210/er.2010-0028). [PubMed: [22240243](https://pubmed.ncbi.nlm.nih.gov/22240243/)].
12. Nouri Saeidlou S, Rezaiegyjeloo F, Ayremlou P, Babaie F. Changes of overweight and obesity prevalence among school children in North West of Iran after 3 years follow-up (2009-2011): A longitudinal study. *Int J Prev Med*. 2016;**7**:79. doi: [10.4103/2008-7802.183651](https://doi.org/10.4103/2008-7802.183651). [PubMed: [27330685](https://pubmed.ncbi.nlm.nih.gov/27330685/)]. [PubMed Central: [PMC4910311](https://pubmed.ncbi.nlm.nih.gov/PMC4910311/)].
13. Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: A follow-up study for up to 20 years. *Gut*. 2009;**58**(11):1538-44. doi: [10.1136/gut.2008.171280](https://doi.org/10.1136/gut.2008.171280). [PubMed: [19625277](https://pubmed.ncbi.nlm.nih.gov/19625277/)]. [PubMed Central: [PMC2792743](https://pubmed.ncbi.nlm.nih.gov/PMC2792743/)].
14. Eshraghian A, Taghavi Seyed A, Nikeghbalian S, Kazemi K, Shamsaeifar A, Mansourian M, et al. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in Iranian patients. *Iran J Gastroenterol Hepatol (Govaresh)*. 2017;**22**(3).
15. El-Koofy N, El-Karakasy H, El-Akel W, Helmy H, Anwar G, El-Sayed R, et al. Ultrasonography as a non-invasive tool for detection of nonalcoholic fatty liver disease in overweight/obese Egyptian children. *Eur J Radiol*. 2012;**81**(11):3120-3. doi: [10.1016/j.ejrad.2012.06.020](https://doi.org/10.1016/j.ejrad.2012.06.020). [PubMed: [22817846](https://pubmed.ncbi.nlm.nih.gov/22817846/)].
16. Alavian SM, Mohammad-Alizadeh AH, Esna-Ashari F, Ardalan G, Hajarizadeh B. Non-alcoholic fatty liver disease prevalence among school-aged children and adolescents in Iran and its association with biochemical and anthropometric measures. *Liver Int*. 2009;**29**(2):159-63. doi: [10.1111/j.1478-3231.2008.01790.x](https://doi.org/10.1111/j.1478-3231.2008.01790.x). [PubMed: [18492015](https://pubmed.ncbi.nlm.nih.gov/18492015/)].
17. Widhalm K, Ghods E. Nonalcoholic fatty liver disease: A challenge for pediatricians. *Int J Obes (Lond)*. 2010;**34**(10):1451-67. doi: [10.1038/ijo.2010.185](https://doi.org/10.1038/ijo.2010.185). [PubMed: [20838401](https://pubmed.ncbi.nlm.nih.gov/20838401/)].
18. Roberts EA. Pediatric nonalcoholic fatty liver disease (NAFLD): A "growing" problem? *J Hepatol*. 2007;**46**(6):1133-42. doi: [10.1016/j.jhep.2007.03.003](https://doi.org/10.1016/j.jhep.2007.03.003). [PubMed: [17445934](https://pubmed.ncbi.nlm.nih.gov/17445934/)].
19. Loomba R, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology*. 2009;**50**(4):1282-93. doi: [10.1002/hep.23119](https://doi.org/10.1002/hep.23119). [PubMed: [19637286](https://pubmed.ncbi.nlm.nih.gov/19637286/)]. [PubMed Central: [PMC2757471](https://pubmed.ncbi.nlm.nih.gov/PMC2757471/)].
20. Denzer C, Thiere D, Mucbe R, Koenig W, Mayer H, Kratzer W, et al. Gender-specific prevalences of fatty liver in obese children and adolescents: Roles of body fat distribution, sex steroids, and insulin resistance. *J Clin Endocrinol Metab*. 2009;**94**(10):3872-81. doi: [10.1210/jc.2009-1125](https://doi.org/10.1210/jc.2009-1125). [PubMed: [19773396](https://pubmed.ncbi.nlm.nih.gov/19773396/)].
21. Felix DR, Costenaro F, Gottschall CB, Coral GP. Non-alcoholic fatty liver disease (Nafld) in obese children- effect of refined carbohydrates in diet. *BMC Pediatr*. 2016;**16**(1):187. doi: [10.1186/s12887-016-0726-3](https://doi.org/10.1186/s12887-016-0726-3). [PubMed: [27846831](https://pubmed.ncbi.nlm.nih.gov/27846831/)]. [PubMed Central: [PMC511335](https://pubmed.ncbi.nlm.nih.gov/PMC511335/)].
22. Ozhan B, Ersoy B, Ozkol M, Kiremitci S, Ergin A. Waist to height ratio: A simple screening tool for nonalcoholic fatty liver disease in obese children. *Turk J Pediatr*. 2016;**58**(5):518-23. doi: [10.24953/turkjped.2016.05.009](https://doi.org/10.24953/turkjped.2016.05.009). [PubMed: [28621093](https://pubmed.ncbi.nlm.nih.gov/28621093/)].
23. Pawar SV, Zanwar VG, Choksey AS, Mohite AR, Jain SS, Surude RG, et al. Most overweight and obese Indian children have non-alcoholic fatty liver disease. *Ann Hepatol*. 2016;**15**(6):853-61. doi: [10.5604/16652681.1222101](https://doi.org/10.5604/16652681.1222101). [PubMed: [27740518](https://pubmed.ncbi.nlm.nih.gov/27740518/)].
24. Nobili V, Alkhouiri N, Alisi A, Della Corte C, Fitzpatrick E, Raponi M, et al. Nonalcoholic fatty liver disease: A challenge for pediatricians. *JAMA Pediatr*. 2015;**169**(2):170-6. doi: [10.1001/jamapediatrics.2014.2702](https://doi.org/10.1001/jamapediatrics.2014.2702). [PubMed: [25506780](https://pubmed.ncbi.nlm.nih.gov/25506780/)].
25. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology*. 2012;**55**(6):2005-23. doi: [10.1002/hep.25762](https://doi.org/10.1002/hep.25762). [PubMed: [22488764](https://pubmed.ncbi.nlm.nih.gov/22488764/)].
26. Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology*. 2010;**52**(3):913-24. doi: [10.1002/hep.23784](https://doi.org/10.1002/hep.23784). [PubMed: [20648476](https://pubmed.ncbi.nlm.nih.gov/20648476/)]. [PubMed Central: [PMC3070295](https://pubmed.ncbi.nlm.nih.gov/PMC3070295/)].
27. Malakouti M, Kataria A, Ali SK, Schenker S. Elevated liver enzymes in asymptomatic patients - what should I do? *J Clin Transl Hepatol*. 2017;**5**(4):394-403. doi: [10.14218/JCTH.2017.00027](https://doi.org/10.14218/JCTH.2017.00027). [PubMed: [29226106](https://pubmed.ncbi.nlm.nih.gov/29226106/)]. [PubMed Central: [PMC5719197](https://pubmed.ncbi.nlm.nih.gov/PMC5719197/)].
28. Bi WR, Yang CQ, Shi Q, Xu Y, Cao CP, Ling J, et al. Large-scale analysis of factors influencing nonalcoholic fatty liver disease and its relationship with liver enzymes. *Genet Mol Res*. 2014;**13**(3):5880-91. doi: [10.4238/2014.August.7.3](https://doi.org/10.4238/2014.August.7.3). [PubMed: [25117346](https://pubmed.ncbi.nlm.nih.gov/25117346/)].
29. Tomizawa M, Kawanabe Y, Shinozaki F, Sato S, Motoyoshi Y, Sugiyama T, et al. Triglyceride is strongly associated with nonalcoholic fatty liver disease among markers of hyperlipidemia and diabetes. *Biomed Rep*. 2014;**2**(5):633-6. doi: [10.3892/br.2014.309](https://doi.org/10.3892/br.2014.309). [PubMed: [25054002](https://pubmed.ncbi.nlm.nih.gov/25054002/)]. [PubMed Central: [PMC4106613](https://pubmed.ncbi.nlm.nih.gov/PMC4106613/)].
30. Zhang S, Du T, Zhang J, Lu H, Lin X, Xie J, et al. The triglyceride and glucose index (TyG) is an effective biomarker to identify nonalcoholic fatty liver disease. *Lipids Health Dis*. 2017;**16**(1):15. doi: [10.1186/s12944-017-0409-6](https://doi.org/10.1186/s12944-017-0409-6). [PubMed: [28103934](https://pubmed.ncbi.nlm.nih.gov/28103934/)]. [PubMed Central: [PMC5248473](https://pubmed.ncbi.nlm.nih.gov/PMC5248473/)].
31. Kim JY, Cho J, Yang HR. Biochemical predictors of early onset non-alcoholic fatty liver disease in young children with obesity. *J Korean Med Sci*. 2018;**33**(16). e122. doi: [10.3346/jkms.2018.33.e122](https://doi.org/10.3346/jkms.2018.33.e122). [PubMed: [29651819](https://pubmed.ncbi.nlm.nih.gov/29651819/)]. [PubMed Central: [PMC5897157](https://pubmed.ncbi.nlm.nih.gov/PMC5897157/)].
32. Cazzo E, Jimenez LS, Gestic MA, Utrini MP, Chaim FHM, Chaim FDM, et al. Type 2 diabetes mellitus and simple glucose metabolism parameters may reliably predict nonalcoholic fatty liver disease features. *Obes Surg*. 2018;**28**(1):187-94. doi: [10.1007/s11695-017-2829-9](https://doi.org/10.1007/s11695-017-2829-9). [PubMed: [28741239](https://pubmed.ncbi.nlm.nih.gov/28741239/)].
33. Roberts EA. Non-alcoholic fatty liver disease (NAFLD) in children. *Front Biosci*. 2005;**10**:2306-18. doi: [10.2741/1699](https://doi.org/10.2741/1699). [PubMed: [15970496](https://pubmed.ncbi.nlm.nih.gov/15970496/)].
34. Kim JS. The role of inflammatory mediators in the pathogenesis of nonalcoholic fatty liver disease. *Pediatric Gastroenterol Hepatol Nutr*. 2012;**15**(2):74. doi: [10.5223/pghn.2012.15.2.74](https://doi.org/10.5223/pghn.2012.15.2.74).
35. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in non-alcoholic fatty liver disease. *Semin Liver Dis*. 2008;**28**(4):360-9. doi: [10.1055/s-0028-1091980](https://doi.org/10.1055/s-0028-1091980). [PubMed: [18956292](https://pubmed.ncbi.nlm.nih.gov/18956292/)]. [PubMed Central: [PMC2908270](https://pubmed.ncbi.nlm.nih.gov/PMC2908270/)].
36. Yki-Jarvinen H. Nutritional modulation of nonalcoholic fatty liver

- disease and insulin resistance: human data. *Curr Opin Clin Nutr Metab Care*. 2010;**13**(6):709-14. doi: [10.1097/MCO.0b013e32833f4b34](https://doi.org/10.1097/MCO.0b013e32833f4b34). [PubMed: [20842026](https://pubmed.ncbi.nlm.nih.gov/20842026/)].
37. Day CP, James OFW. Steatohepatitis: A tale of two "hits"? *Gastroenterology*. 1998;**114**(4):842-5. doi: [10.1016/s0016-5085\(98\)70599-2](https://doi.org/10.1016/s0016-5085(98)70599-2).
38. Sheng X, Che H, Ji Q, Yang F, Lv J, Wang Y, et al. The relationship between liver enzymes and insulin resistance in type 2 diabetes patients with nonalcoholic fatty liver disease. *Horm Metab Res*. 2018;**50**(5):397-402. doi: [10.1055/a-0603-7899](https://doi.org/10.1055/a-0603-7899). [PubMed: [29723898](https://pubmed.ncbi.nlm.nih.gov/29723898/)].
39. Kaltenbach TE, Graeter T, Oeztuerk S, Holzner D, Kratzer W, Wabitsch M, et al. Thyroid dysfunction and hepatic steatosis in overweight children and adolescents. *Pediatr Obes*. 2017;**12**(1):67-74. doi: [10.1111/ijpo.12110](https://doi.org/10.1111/ijpo.12110). [PubMed: [26877190](https://pubmed.ncbi.nlm.nih.gov/26877190/)]. [PubMed Central: [PMC5248640](https://pubmed.ncbi.nlm.nih.gov/PMC5248640/)].
40. Bernhardt P, Kratzer W, Schmidberger J, Graeter T, Gruener B, Emil Study Group. Laboratory parameters in lean NAFLD: comparison of subjects with lean NAFLD with obese subjects without hepatic steatosis. *BMC Res Notes*. 2018;**11**(1):101. doi: [10.1186/s13104-018-3212-1](https://doi.org/10.1186/s13104-018-3212-1). [PubMed: [29409538](https://pubmed.ncbi.nlm.nih.gov/29409538/)]. [PubMed Central: [PMC5801753](https://pubmed.ncbi.nlm.nih.gov/PMC5801753/)].
41. Chung GE, Kim D, Kim W, Yim JY, Park MJ, Kim YJ, et al. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. *J Hepatol*. 2012;**57**(1):150-6. doi: [10.1016/j.jhep.2012.02.027](https://doi.org/10.1016/j.jhep.2012.02.027). [PubMed: [22425701](https://pubmed.ncbi.nlm.nih.gov/22425701/)].
42. Lee J, Ha J, Jo K, Lim DJ, Lee JM, Chang SA, et al. Male-specific association between subclinical hypothyroidism and the risk of non-alcoholic fatty liver disease estimated by hepatic steatosis index: Korea National Health and Nutrition Examination Survey 2013 to 2015. *Sci Rep*. 2018;**8**(1):15145. doi: [10.1038/s41598-018-32245-0](https://doi.org/10.1038/s41598-018-32245-0). [PubMed: [30310098](https://pubmed.ncbi.nlm.nih.gov/30310098/)]. [PubMed Central: [PMC6181925](https://pubmed.ncbi.nlm.nih.gov/PMC6181925/)].