




Non-alcoholic Fatty Liver Disease in Overweight and Obese Children; Can Ultrasonography Substitute Laboratory Studies? A Case-Control Study

Abbas Avazpour¹, Mohadeseh Bahramnejad ^{1, *}, Amir Hossein Hassani², Sara Matin¹, Maryam Moatamedi¹

¹ Department of Pediatrics, Jahrom University of Medical Sciences, Jahrom, Iran

² School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author: Department of Pediatrics, Jahrom University of Medical Sciences, Jahrom, Iran. Email: mohadesebahramnejad@gmail.com

Received 2024 April 23; Revised 2024 June 12; Accepted 2024 June 24.

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) comprises a large burden on the global healthcare system with a high prevalence and lethal outcomes. Moreover, this disease is showing an incremental trend over time, partly due to the rise in obesity rates among children.

Objectives: In this study, we aimed to elucidate the correlation of ultrasonographic and laboratory-assisted diagnosis of NAFLD in obese and normal-weight children.

Methods: This study assessed 50 pediatric obese or overweight children with BMIs higher than the 85th percentile for their age and sex, as well as 50 age- and sex-matched control counterparts using ultrasonography and a liver enzyme panel. The prevalence of NAFLD and the differences in lab studies were evaluated. Then, receiver operating characteristics (ROC) curves were used to determine the optimal cut-off value for NAFLD based on liver enzymes.

Results: A total of 100 participants were included in this study, 58% of whom were male. The mean age of the participants was 10.41 ± 2.224 years. The prevalence of sonographically diagnosed NAFLD in obese and overweight children was 34.0% (95% CI: 21.2% - 48.8%). The prevalence of NAFLD when assessed by lab values in this population was 58.0% (95% CI: 43.2% - 71.8%), with a low measure of agreement ($K = 0.239$, $P = 0.058$). High BMI increased the risk of NAFLD to about 8.5 times its baseline in the normal-BMI population. Non-alcoholic fatty liver disease patients had significantly higher values for aspartate aminotransferase (AST) ($P < 0.001$), alanine aminotransferase (ALT) ($P < 0.001$), and gamma-glutamyl transferase (GGT) ($P < 0.001$), but not alkaline phosphatase (ALP) ($P = 0.743$). The ROC curves for AST, ALT, GGT, and BMI percentile had areas of 0.800, 0.899, 0.807, and 0.874, respectively, all of which could be used for screening NAFLD. A cut-off value of 43.5 IU/L for AST had a specificity and positive predictive value of 100% for the diagnosis of NAFLD in children.

Conclusions: Obese and overweight children are 8.5 times more likely to develop NAFLD compared to their normal-BMI peers. For the evaluation of this disease, liver enzyme panels integrated with ultrasonographic assessment should be utilized.

Keywords: Non-alcoholic Fatty Liver Disease, Ultrasonography, Pediatric Obesity

1. Background

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic hepatologic disorder, affecting nearly 38.0% of the global population (1). Non-alcoholic fatty liver disease is characterized by the accumulation of lipids in hepatic cells, known as steatosis (2). If left untreated, NAFLD can progress to Non-alcoholic steatohepatitis (NASH), marked by inflammation and fat

infiltration of the liver parenchyma, potentially leading to liver cell apoptosis (3). Non-alcoholic steatohepatitis can then result in fatal conditions such as hepatocellular carcinoma (HCC), hepatic fibrosis, and cirrhosis (4). Despite extensive research on the epidemiology of NAFLD and NASH in adults, data on the pediatric population is limited. However, it is evident that the incidence of NAFLD/NASH among children has been steadily increasing by approximately 1.3% annually,

with the highest rates seen in the Middle East (5). This rise in pediatric NAFLD/NASH cases is likely linked to the growing prevalence of childhood obesity and the adoption of a Western diet high in fats and simple carbohydrates, and low in fibers (6).

As NASH is now the second most common reason for liver transplantation in developed countries, it is crucial to prioritize preventative measures, such as early treatment of NAFLD, to address this issue (7). While pharmacological treatments for NAFLD and NASH have not yielded significant results, emphasizing lifestyle modifications and prevention remains key (8). Addressing obesity and improving diet are primary steps in treating and preventing NAFLD/NASH (9).

Various studies have proposed serologic, histologic, and radiologic parameters for diagnosing pediatric NAFLD. Biomarkers such as cytokeratin 18, Fas, Fas ligand, and oxidized low-density lipoprotein have shown elevated levels in NAFLD patients compared to healthy individuals. However, these biomarkers require specialized laboratory equipment that may not be readily available, particularly in resource-limited settings (10). Histological evaluation of liver tissue and assessment of hepatic fibrosis through liver biopsy are crucial for determining mortality risk (11). Alternatively, ultrasound evaluation of the liver parenchyma compared to the right renal parenchyma can provide a highly sensitive and specific diagnosis of NAFLD. In fact, European guidelines recommend ultrasound as the primary diagnostic tool for NAFLD in adults (12, 13). The effectiveness of ultrasound in diagnosing NAFLD in obese children versus normal-weight children requires further investigation.

2. Objectives

This study aims to compare ultrasonographic findings with traditional liver enzyme tests for diagnosing NAFLD in both normal-weight and obese children.

3. Methods

This study followed a case-control protocol and was conducted in Jahrom, southern Iran, on 100 children who attended the Honari clinic of Jahrom University of Medical Sciences. After explaining the course of the study and obtaining written informed consent from the patients' parents, 50 obese or overweight children and 50 non-obese, age- and sex-matched children underwent ultrasonography and serum biochemical testing to evaluate liver enzymes.

The inclusion criteria of our study were children aged 5 - 18 years with no signs or symptoms of extensive liver failure and no history of consumption of steatogenic compounds. The exclusion criteria were unwillingness of the parents to participate in the study, cirrhosis, or signs and symptoms of this condition including ascites, jaundice, esophageal varices, or splenomegaly. Patients with viral hepatitis, celiac disease, hypothyroidism, Wilson's disease, autoimmune hepatitis, α -antitrypsin deficiency, and abetalipoproteinemia were also excluded.

Obesity or overweight in children was defined as a Body Mass Index (BMI) higher than the 85th percentile for their age according to the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) (14, 15). Weight was measured by a single digital weighing scale with a precision of 0.1 kg, and height was measured by a single stadiometer in a posture where the patients' shoulders, heels, and buttocks touched the wall and the head kept horizontally with a precision of 0.1 cm.

In addition to the anthropometric evaluation, five milliliters of blood was taken from each participant for the measurement of gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using an auto-analyzer (BT1500; Biotecnica instrument) and Pars Azmun® standardized kits.

The patients then underwent comprehensive abdominopelvic ultrasonography by a single expert radiologist for the assessment of fatty liver and its grading. Grade I fatty liver was defined as diffusely increased hepatic parenchymal echogenicity with significant periportal and diaphragmatic echogenicity while in grade II fatty liver, periportal echogenicity is obscured. In grade III fatty liver, although the hepatic echogenicity is increased, the diaphragmatic and periportal echogenicity are both obscured (16).

The diagnosis of NAFLD was also made by the use of liver enzymes as suggested by the North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) with serum ALT levels above 50 U/L for boys and above 44 U/L for girls as twice the upper limit of normal (17).

The data were entered into IBM SPSS version 24.0. Qualitative variables were reported as frequency and percentages, and quantitative variables were reported as mean and standard deviation. The prevalence of NAFLD was calculated in each group by the defining characteristics of ultrasonography and lab values, and the 95% confidence interval for each prevalence rate was estimated using a one-sample binomial test. The Kappa

measure of agreement was used to assess the two diagnostic methods, and Spearman's correlation test was used to evaluate the correlation of ultrasonographic grading of NAFLD with lab values. Receiver operating characteristics (ROC) curves were drawn for each lab value based on the diagnosis of NAFLD using ultrasonography to find the best cut-off values for these values for the diagnosis of NAFLD. P-values less than 0.05 were considered statistically significant.

This study was conducted in accordance with the Helsinki declaration on research ethics. It was also approved by the Committee of Ethics in Biomedical Research of Jahrom University of Medical Sciences with the code: [IR.JUMS.REC.1403.001](#).

4. Results

A total of 100 participants entered the study, half of whom were overweight or obese. The other half were age- and sex-matched counterparts with a normal BMI. In this study, 58 participants (58.0%) were male and 42 participants (42.0%) were female. The mean age of the participants was 10.41 ± 2.224 years. The patients who were overweight or obese had higher liver enzyme and gamma-glutamyl transferase levels. [Table 1](#) summarizes the baseline demographic and clinical information of the participants in our study.

The patients underwent comprehensive abdominopelvic ultrasonography, which revealed a prevalence of 34% (95% CI: 21.2% - 48.8%) for NAFLD in the case group and a prevalence of 4.0% (95% CI: 0.5% - 13.7%) in the control group. In the assessment of NAFLD grading, overweight and obese participants experienced higher severities of NAFLD compared to their normal BMI counterparts. The diagnosis of NAFLD was again made by the lab data with a cutoff value of 50 IU/L for ALT and AST, resulting in a prevalence of 58.0% for NAFLD in overweight and obese children. The kappa measure of agreement was calculated for both groups to evaluate the agreement of sonographic and laboratory diagnosis of NAFLD. In obese children, the kappa measure of agreement was only 0.239 and non-significant, but this measurement in normal-BMI children was 1.000 and significant. [Table 2](#) summarizes the comparison of the two groups regarding the prevalence of NAFLD.

In this study population, liver enzymes and gamma-glutamyl transferase were compared among the patients with and without NAFLD. In obese children, patients with NAFLD had significantly higher AST levels (43.06 ± 21.510 vs. 29.96 ± 8.605 , $P = 0.015$), an association that was also seen in the total population. In obese children and the total population, NAFLD patients also

had significantly higher ALT levels (41.72 ± 18.464 vs. 72.29 ± 35.721 , $P < 0.001$ in the obese children and 26.03 ± 17.584 vs. 70.47 ± 34.248 , $P < 0.001$ in the total population). The association between GGT and NAFLD was observed in obese children and the total population as well ($P = 0.025$ and $P < 0.001$, respectively). [Table 3](#) summarizes the association between NAFLD and laboratory values.

For the assessment of the sonographic grading of NAFLD and laboratory studies in children, Spearman's correlation was utilized, which showed a significant direct correlation between AST, ALT, GGT, and BMI percentile with NAFLD grading in overweight and obese children ($P = 0.011$, 0.001 , 0.007 , and < 0.001 , respectively) and a direct correlation between the same variables with NAFLD grading in the total population ($P < 0.001$ for all four lab values). [Table 4](#) summarizes the correlation between NAFLD grading and lab studies.

Furthermore, ROC curves for the lab values were drawn for the total population and obese children. [Figure 1](#) shows the ROC curves of the diagnosis of NAFLD based on various lab values. The curves for AST, ALT, GGT, and BMI percentile were significant for the prediction of NAFLD. Aspartate aminotransferase seemed to be the most superior lab value to evaluate in patients suspected of NAFLD, as in obese children and in the total population, AST with a cut-off value of 43.5 had 100% specificity and positive predictive value for NAFLD. The optimal cut-off values of the lab values are summarized in [Table 5](#).

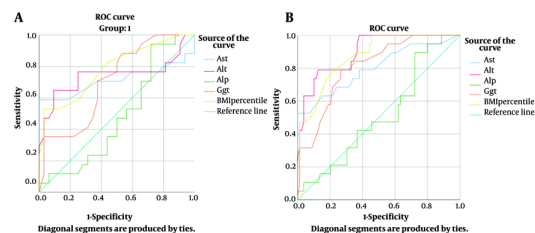


Figure 1. Receiver operating characteristic (ROC) curves for diagnosis of non-alcoholic Fatty Liver Disease (NAFLD) based on liver enzyme

5. Discussion

In this study, a total of 100 children, half of whom were obese or overweight, were evaluated regarding their liver enzymes and sonographic evidence of NAFLD. We found that the prevalence of NAFLD in normal-BMI children was 4.0% (95% CI: 0.5% - 13.7%), while the prevalence of this disease among overweight and obese

Table 1. Baseline Demographic and Clinical Information of the Study Population ^a

Variables	Cases	Controls	P-Value
Sex			> 0.99
Male	29 (58.0)	29 (58.0)	
Female	21(42.0)	21(42.0)	
Age	10.41 ± 2.224	10.41 ± 2.224	> 0.99
BMI percentile			
Lab studies	89.40 ± 3.326	44.08 ± 21.534	< 0.001
AST	34.24 ± 15.663	11.08 ± 1.766	< 0.001
ALT	52.33 ± 29.355	16.96 ± 8.517	< 0.001
ALP	654.90 ± 258.390	599.14 ± 238.905	0.140
GGT	25.74 ± 8.278	16.36 ± 6.090	< 0.001

Abbreviations: BMI: Body Mass Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase.

^a Values are presented as No. (%) or mean ± SD.

Table 2. Prevalence of Non-alcoholic Fatty Liver Disease (NAFLD) in Obese Children and Normal-BMI Children

Non-alcoholic Fatty Liver Disease (NAFLD)	Case Group					Control Group				
	Sonographic		Lab Data		K Measure	Sonographic		Lab Data		K Measure
N (%)	95%CI	N (%)	95%CI	N (%)	95%CI	N (%)	95%CI			
Total	17 (34.0)	21.2 - 48.8	29 (58.0)	43.2 - 71.8	0.239 (P = 0.058)	2 (4.0)	0.5 - 13.7	2 (4.0)	0.5 - 13.7	1.000 (P < 0.001)
Mild	12 (24.0)	13.1 - 38.2	-	-	-	2 (4.0)	0.5 - 13.7	-	-	-
Moderate	3 (6.0)	1.3 - 16.5	-	-	-	0 (0.0)	0.0 - 7.1	-	-	-
Severe	2 (4.0)	0.5 - 13.7	-	-	-	0 (0.0)	0.0 - 7.1	-	-	-

Table 3. Comparison of Liver Enzyme Panel in NAFLD Patients and Their Healthy Counterparts ^a

Variables	NAFLD vs. no NAFLD in Obese Children			NAFLD vs. no NAFLD in Total Population		
	No NAFLD	NAFLD	P-Value	No NAFLD	NAFLD	P-Value
AST	29.96 ± 8.605	43.06 ± 21.510	0.004	18.68 ± 10.907	39.89 ± 22.385	< 0.001
ALT	41.72 ± 18.464	72.29 ± 35.721	< 0.001	26.03 ± 17.584	70.47 ± 34.248	< 0.001
ALP	655.00 ± 279.646	669.41 ± 225.976	0.845	625.81 ± 257.087	645.35 ± 225.762	0.743
GGT	22.97 ± 7.577	28.65 ± 6.982	0.025	18.91 ± 7.422	27.95 ± 7.291	< 0.001

Abbreviations: NAFLD, non-alcoholic fatty liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase.

^a Values are presented as mean ± SD.

children was 34.0% (95% CI: 21.2% - 48.8%). This indicates that overweight and obese children had a relative risk of about 8.5 for the development of NAFLD. In the global pediatric population, the prevalence of NAFLD is estimated to be between five and ten percent (18). Considering a national prevalence of about 11% for childhood obesity (19), the findings of our study align with previous studies evaluating the prevalence of NAFLD in children. Since pediatric NAFLD patients are at higher risk for cardiovascular mortality later in life, thorough evaluation of this condition and proper

management of this disease and its sequelae seem to be important for healthcare policymakers (20).

Since the gold standard for diagnosing NAFLD and NASH consists of the invasive procedure of a liver biopsy, finding an alternative to this approach would increase the willingness of patients to undergo evaluation for this condition. Non-invasive diagnostic tools can also act as screening measures that suggest further steps in the NAFLD work-up. For this purpose, ultrasonography has been widely used, as it can show fatty infiltration of the hepatic parenchyma (21). However, the association

Table 4. Correlation of Non-alcoholic Fatty Liver Disease (NAFLD) Grade with Liver Enzyme Panel

Variables	NAFLD Grade in Obese Children		NAFLD Grade in Total Population	
	Spearman's ρ	P-Value	Spearman's ρ	P-Value
AST	0.362	0.011	0.413	< 0.001
ALT	0.446	0.001	0.554	< 0.001
ALP	- 0.79	0.587	- 0.013	0.901
GGT	0.379	0.007	0.427	< 0.001
BMI percentile	0.527	< 0.001	0.522	< 0.001

Abbreviations: NAFLD, non-alcoholic fatty liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; BMI, Body Mass Index.

Table 5. Optimal Cut-off Values of Liver Enzyme Panel for Diagnosis of Non-alcoholic Fatty Liver Disease (NAFLD) in Children ^a

Variables	Obese Children							Total Population						
	AUC	P-Value	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P-Value	Cut-off	Sensitivity	Specificity	PPV	NPV
AST	0.713	0.015	43.5	58.8	100	100	82.5	0.800	< 0.001	43.5	52.6	100	100	90
ALT	0.744	0.005	65.0	64.7	91.6	78.6	83.3	0.899	< 0.001	62.5	63.2	96.2	80	91.8
ALP	0.483	0.842	N/A	N/A	N/A	N/A	N/A	0.509	0.907	N/A	N/A	N/A	N/A	N/A
GGT	0.705	0.019	33.5	35.3	96.9	85.7	74.4	0.807	< 0.001	29.5	31.6	91.1	46.2	85.1
BMI percentile	0.784	0.001	92.5	52.9	96.9	90.0	80.0	0.874	< 0.001	90.5	47.4	98.7	75.0	88.6

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; BMI, Body Mass Index.

^a Values are presented as No. (%).

between sonographic findings and lab studies has not been comprehensively studied. In this study, we used ultrasonography and laboratory studies as diagnostic methods for NAFLD. In the overweight and obese population, the agreement between the two methods was poor. The patients with NAFLD showed higher levels of alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase enzymes. The difference between NAFLD patients and normal children was more prominent when normal-BMI and high - BMI children were included in the comparison. This might stem from the fact that such enzymes might be influenced by other conditions seen in obesity, not only NAFLD (22). For example, obese and overweight children are more likely to have obesity-related hormonal disorders, such as polycystic ovary syndrome, which increases ALT levels (23, 24). Furthermore, the adipose tissue in obese children secretes pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), leptin, resistin, and adipokines, all of which increase oxidative stress in the hepatic parenchyma, leading to increased liver enzymes (25).

In the assessment of the correlation between lab studies and the degree of NAFLD, Spearman's correlation test was used. The most important

correlating factors were BMI percentile ($\rho = 0.527$, $P < 0.001$ in the total population, and $\rho = 0.522$, $P < 0.001$ in obese children) and ALT ($\rho = 0.446$, $P = 0.001$ in the total population and $\rho = 0.554$, $P < 0.001$ in obese children). In a study by Mansour-Ghanaei et al., performed on 950 participants of the PERSIAN Guilan cohort study, they assessed the association between liver enzymes and NAFLD and found that ALT, AST, and GGT were associated with this condition, with ALT being the most strongly correlated factor. ALP was not associated with this condition (26). The findings of our study align with those of the PERSIAN Guilan cohort study.

In a study by Khodadoostan et al., performed on 109 adult patients with NAFLD, the results of liver biopsy were compared with liver enzymes and ultrasonography findings. They found that the steatosis level in histopathology was associated with sonography findings, but a direct correlation was not found between liver enzymes and the steatosis level. They proposed that sonographic findings, unlike liver enzymes, might be useful in screening patients with NAFLD (27). These findings align with our results.

We also evaluated the optimal cutoff points for each laboratory parameter for the diagnosis of NAFLD. In this study, the BMI percentile outperformed other variables by having the highest area under the curve (AUC).

However, a cutoff value of 43 IU/mL for AST had a positive predictive value of 100% for the diagnosis of NAFLD, making it more practical. Multiple models have incorporated various parameters like the liver function panel to evaluate the diagnosis of NAFLD. For example, Palekar et al. used AST, the AST/ALT ratio, BMI, age, and fasting insulin for a model that had an AUC of 0.76 (28). Angulo et al. also used age, history of hyperglycemia, BMI, platelet count, albumin levels, and the AST/ALT ratio for the diagnosis of liver fibrosis following NAFLD (29). In our study, the liver panel alone could indicate NAFLD with good precision.

The main limitation of this study was the relatively low number of patients and the inability to perform more advanced imaging like magnetic resonance elastography. Additionally, we could not correlate the results of sonography with liver fibrosis as assessed by histology. For further research, prospective studies with liver biopsy evaluations are suggested to measure the sensitivity, specificity, positive predictive value, and negative predictive value of sonography.

5.1. Conclusions

In this study, the prevalence of NAFLD in the normal-BMI pediatric population was 4.0%, but overweight and obese children were 8.5 times more likely to have NAFLD. Pediatric patients with sonographically diagnosed NAFLD had significantly higher levels of ALT, AST, and GGT, but no association was found between NAFLD and alkaline phosphatase. There was a low kappa measure of agreement between the sonographic and laboratory diagnosis of NAFLD in children, especially in obese and overweight patients. Implementing only lab values as a screening test for NAFLD seems unrealistic; therefore, sonographic evaluations should be integrated into the diagnostic evaluations of such patients.

Footnotes

Authors' Contribution: A.A, designed the study; M.B., A.H.H., S.M., and M.M. gathered the data; A.H.H., analyzed the data; M.B. and A.H.H., prepared the first draft of the manuscript. All of the authors have read the manuscript and made the necessary revisions. The final manuscript is accepted by all of the authors.

Conflict of Interests Statement: The authors declare no conflicts of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to patient confidentiality.

Ethical Approval: This study was conducted in accordance with Helsinki declaration on research ethics. This study was also approved by the committee of ethics in biomedical research of Jahrom University of Medical Sciences by the code: [IR.JUMS.REC.1403.001](https://doi.org/10.1097/JHEP.0000000000000004).

Funding/Support: The study was funded by Jahrom University of Medical Sciences as the dissertation of MB for specialty in pediatrics.

Informed Consent: Informed consent was obtained from the parents of the participants.

References

1. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*. 2023;77(4):1335-47. [PubMed ID: 36626630]. [PubMed Central ID: PMC10026948]. <https://doi.org/10.1097/JHEP.0000000000000004>.
2. Nassir F. NAFLD: Mechanisms, Treatments, and Biomarkers. *Biomolecules*. 2022;12(6). [PubMed ID: 35740949]. [PubMed Central ID: PMC9221336]. <https://doi.org/10.3390/biom12060824>.
3. Raza S, Rajak S, Upadhyay A, Tewari A, Anthony Sinha R. Current treatment paradigms and emerging therapies for NAFLD/NASH. *Front Biosci (Landmark Ed)*. 2021;26(2):206-37. [PubMed ID: 33049668]. [PubMed Central ID: PMC7116261]. <https://doi.org/10.2741/4892>.
4. Raza S, Rajak S, Anjum B, Sinha RA. Molecular links between non-alcoholic fatty liver disease and hepatocellular carcinoma. *Hepatoma Res*. 2019;5:42. [PubMed ID: 31867441]. [PubMed Central ID: PMC6924993]. <https://doi.org/10.20517/2394-5079.2019.014>.
5. Zhang X, Wu M, Liu Z, Yuan H, Wu X, Shi T, et al. Increasing prevalence of NAFLD/NASH among children, adolescents and young adults from 1990 to 2017: a population-based observational study. *BMJ Open*. 2021;11(5). e042843. [PubMed ID: 33947727]. [PubMed Central ID: PMC8098935]. <https://doi.org/10.1136/bmjopen-2020-042843>.
6. Shaunak M, Byrne CD, Davis N, Afolabi P, Faust SN, Davies JH. Non-alcoholic fatty liver disease and childhood obesity. *Arch Dis Child*. 2021;106(1):3-8. [PubMed ID: 32409495]. <https://doi.org/10.1136/archdischild-2019-318063>.
7. Younossi ZM, Stepanova M, Ong J, Trimble G, AlQahtani S, Younossi I, et al. Nonalcoholic Steatohepatitis Is the Most Rapidly Increasing Indication for Liver Transplantation in the United States. *Clin Gastroenterol Hepatol*. 2021;19(3):580-589 e5. [PubMed ID: 32531342]. <https://doi.org/10.1016/j.cgh.2020.05.064>.
8. Muthiah MD, Sanyal AJ. Current management of non-alcoholic steatohepatitis. *Liver Int*. 2020;40 Suppl 1(Suppl 1):89-95. [PubMed ID: 32077609]. [PubMed Central ID: PMC7654093]. <https://doi.org/10.1111/liv.14355>.
9. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology*. 2010;51(2):679-89. [PubMed ID: 20041406]. [PubMed Central ID: PMC3575093]. <https://doi.org/10.1002/hep.23280>.
10. Papachristodoulou A, Kavvadas D, Karamitsos A, Papamitsou T, Chatzidimitriou M, Sioga A. Diagnosis and Staging of Pediatric Non-Alcoholic Fatty Liver Disease: Is Classical Ultrasound the Answer? *Pediatr Rep*. 2021;13(2):312-21. [PubMed ID: 34201230]. [PubMed Central ID: PMC8293345]. <https://doi.org/10.3390/pediatric13020039>.
11. Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology*. 2015;61(5):1547-54. [PubMed ID: 25125077]. <https://doi.org/10.1002/hep.27368>.

12. Yu Q, Liu Y, Hu P, Gao F, Huang G. Performance of Imaging Techniques in Non-invasive Diagnosis of Non-alcoholic Fatty Liver Disease in Children: A Systematic Review and Meta-Analysis. *Front Pediatr*. 2022;**10**:837116. [PubMed ID: 35899133]. [PubMed Central ID: PMC9311375]. <https://doi.org/10.3389/fped.2022.837116>.
13. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. 2016;**64**(6):1388-402. [PubMed ID: 27062661]. <https://doi.org/10.1016/j.jhep.2015.11.004>.
14. World Health Organization. *WHO child growth standards: length/height - for - age, weight - for - age, weight - for - length, weight - for - height and body mass index - for - age: methods and development*. Geneva, Switzerland: World Health Organization; 2006, [cited 2023]. Available from: <https://www.who.int/publications/i/item/924154693X>.
15. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. *Adv Data*. 2000;(314):1-27. [PubMed ID: 11183293].
16. Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. *World J Gastroenterol*. 2019;**25**(40):6053-62. [PubMed ID: 31686762]. [PubMed Central ID: PMC6824276]. <https://doi.org/10.3748/wjg.v25.i40.6053>.
17. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr*. 2017;**64**(2):319-34. [PubMed ID: 28107283]. [PubMed Central ID: PMC5413933]. <https://doi.org/10.1097/MPG.0000000000001482>.
18. Yu EL, Schwimmer JB. Epidemiology of Pediatric Nonalcoholic Fatty Liver Disease. *Clin Liver Dis (Hoboken)*. 2021;**17**(3):196-9. [PubMed ID: 33868665]. [PubMed Central ID: PMC8043694]. <https://doi.org/10.1002/cld.1027>.
19. Akbari H, Mohammadi M. The Prevalence of Obesity in Iranian Children: A Systematic Review and Meta-analysis. *J Pediatr Rev*. 2022;**10**(2):93-102. <https://doi.org/10.32598/jpr.10.2.875.2>.
20. 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. [PubMed ID: 19625277]. [PubMed Central ID: PMC2792743]. <https://doi.org/10.1136/gut.2008.171280>.
21. Petzold G. Role of Ultrasound Methods for the Assessment of NAFLD. *J Clin Med*. 2022;**11**(15). [PubMed ID: 35956196]. [PubMed Central ID: PMC9369745]. <https://doi.org/10.3390/jcm11154581>.
22. Jalili V, Poorahmadi Z, Hasanpour Ardekanizadeh N, Gholamalizadeh M, Ajami M, Houshiarrad A, et al. The association between obesity with serum levels of liver enzymes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase in adult women. *Endocrinol Diabetes Metab*. 2022;**5**(6). e367. [PubMed ID: 36039792]. [PubMed Central ID: PMC9659656]. <https://doi.org/10.1002/edm2.367>.
23. Economou F, Xyrafis X, Livadas S, Androurakis J, Argyrakopoulou G, Christakou CD, et al. In overweight/obese but not in normal-weight women, polycystic ovary syndrome is associated with elevated liver enzymes compared to controls. *Hormones (Athens)*. 2009;**8**(3):199-206. [PubMed ID: 19671519]. <https://doi.org/10.14310/horm.2002.1236>.
24. Schwimmer JB, Khorram O, Chiu V, Schwimmer WB. Abnormal aminotransferase activity in women with polycystic ovary syndrome. *Fertil Steril*. 2005;**83**(2):494-7. [PubMed ID: 15705403]. <https://doi.org/10.1016/j.fertnstert.2004.08.020>.
25. Wree A, Kahraman A, Gerken G, Canbay A. Obesity affects the liver - the link between adipocytes and hepatocytes. *Digestion*. 2011;**83**(1-2):124-33. [PubMed ID: 21042023]. <https://doi.org/10.1159/000318741>.
26. Mansour-Ghanaei R, Mansour-Ghanaei F, Naghipour M, Joukar F. Biochemical markers and lipid profile in nonalcoholic fatty liver disease patients in the PERSIAN Guilan cohort study (PGCS), Iran. *J Family Med Prim Care*. 2019;**8**(3):923-8. [PubMed ID: 31041226]. [PubMed Central ID: PMC6482810]. https://doi.org/10.4103/jfmpc.jfmpc_243_18.
27. Khodadoostan M, Shariatifar B, Motamedi N, Abdolahi H. Comparison of liver enzymes level and sonographic findings value with liver biopsy findings in nonalcoholic fatty liver disease patients. *Adv Biomed Res*. 2016;**5**:40. [PubMed ID: 27099853]. [PubMed Central ID: PMC4815528]. <https://doi.org/10.4103/2277-9175.178785>.
28. Palekar NA, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. *Liver Int*. 2006;**26**(2):151-6. [PubMed ID: 16448452]. <https://doi.org/10.1111/j.1478-3231.2005.01209.x>.
29. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatol*. 2007;**45**(4):846-54. [PubMed ID: 17393509]. <https://doi.org/10.1002/hep.21496>.