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OPEN PEER REVIEW

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

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1 Original submission

1 comment · 27 May 2024

Associate Editor

MAJOR REVISION 27 May 2024

Dear Author
Please clarify the novelty of the manuscript.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear reviewers of IJ pediatrics

Thank you for considering our manuscript encoded 148096 for publication. The respected reviewers made some well-deserved points and the following alterations were made to the manuscript.

Reviewer 1:

1. The title is focusing on ultrasound in detection of NAFLD, but there is just few paragraphs in your manuscript explaining about it, the title should be changed, or the article be written based on ultrasound mostly.

   Response: the title of the manuscript is not changed, but the alterations to the manuscript are made to better suit the title.

2. as you know there are many articles regarding this topic, please explain about the novelty of your manuscript.

   Response: the final paragraph of the introduction is added which also states the novelty of the manuscript.

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 were shown to 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. Therefore, this study, aims to compare ultrasonographic findings with traditional liver enzyme tests for diagnosing NAFLD in both normal-weight and obese children.

3. No data is available regarding correlation between grading of fatty liver on ultrasound and the level of AST, ALT.

   Response: the fourth paragraph and table of the results section indicated the correlation of AST and ALT with sonographic grading of NAFLD:

For 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.

Table 4: correlation of NAFLD grade with liver enzyme panel

variable	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

NAFLD: Non-Alcoholic Fatty Liver Disease, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma Glutamyl Transferase, BMI: Body Mass Index

4. please in discussion, compare your findings with other articles which are in line and also against your manuscript.

Response: Paragraphs are added to the discussion for this matter.

In the assessment of the correlation between the results of lab studies with the degree of NAFLD, Spearman's correlation test was used. The most important correlating factors in this regard were BMI percentile (ρ=0.527, P<0.001 in the total population, and ρ=0.522, P<0.001 in obese children) and ALT (ρ=0.446, P=0.001 in the total population and ρ=0.554, P<0.001 in obese children). In a study by Mansour-Ghanaei et al. performed on 950 participants of 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 are in line with the findings 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 steatosis level. They then proposed that sonographic findings, unlike liver enzymes, might be of use in screening of patients with NAFLD [27]. These findings are in line with our results.

5. n conclusion section, nothing is mentioned about ultrasound

response: the conclusion is rewritten to better suit the title.

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

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.

Reviewer 2:

With regard this article is suitable

Response: thank you for your kind review

Reviewer 3:

1. The introduction section needs more comprehensive review of the former literature. Additionally, it is crucial to highlight the novelty of current study and how it contributes to and complements the earlier works.

   Response: the final paragraph of the introduction is added to the manuscript to serve this goal.

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 were shown to 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. Therefore, this study, aims to compare ultrasonographic findings with traditional liver enzyme tests for diagnosing NAFLD in both normal-weight and obese children.

2. The term Non-Alcoholic Steatohepatitis (NASH) has been repeated frequently in this section, even though the rest of the manuscript focuses on NAFLD and does not discuss other related terms.

   Response: the terminology of the manuscript is corrected and unanimous.

3. Given that the inclusion criteria for the study specified children between the ages of 5 and 18, there is no need to mention children under five years old in the exclusion criteria

   Response: children younger than 5 years old has been deleted from the exclusion criteria.

4. n the analysis section, all analysis methods should be included, such as the ROC curve test and cut-offs.

Response: the statistical analysis section of the methods is edited to consist all of the analysis methods.

The data were entered into IBM SPSS version 24.0. The qualitative variables were reported as frequency and percentages, and the 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. 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 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 diagnosis of NAFLD. P-values less than 0.05 were considered statistically significant.

5. Improving the English editing can enhance the paper (e.g. the first paragraph of the results section).

Response: the whole manuscript is revised linguistically for better readability.

6. The statement you provided in abstract and main text is partially wrong from a statistical viewpoint: The prevalence of NAFLD in normal-BMI children is reported as 4.0% with a 95% confidence interval (CI) of 0.5%-13.7%. 1. The prevalence of NAFLD among overweight and obese children is reported as 34.0% with a 95% CI of 21.2%-48.8%. The relative risk (RR) is calculated as the ratio of the probability of an event (in this case, developing NAFLD) in the exposed group (overweight and obese children) compared to the unexposed group (normal-BMI children). The formula for calculating relative risk is: RR= Prevalence of NAFLD among overweight and obese children/Prevalence of NAFLD among normal-BMI children Using the reported prevalence values: RR=34.0%/4.0%≈8.5 Therefore, the corrected statement should say that overweight and obese children had a relative risk of approximately 8.5 for the development of NAFLD compared to normal-BMI children, rather than 17.0 as originally stated. It's important to ensure that statistical calculations, such as relative risk, are accurately computed to convey the correct information and interpretation of the data.

Response: this was a mistake made in our writing and the mistake is revised in the whole manuscript. Thank you for thoroughly investigating the article.

7. The final paragraph of the discussion does not related to the results of the current study. Discussing low-fat or carbohydrate diets is not relevant to the issues and topics investigated in this study.

Response: the paragraph regarding the diet and NAFLD is now deleted from the manuscript

8. Mention to the current studies limitations.

Response: the final paragraph of the discussion is added to serve this goal.

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 and also the inability to correlate the results of sonography with liver fibrosis as assessed by histology. For further research, prospective studies with liver biopsy evaluations are suggested so that the sensitivity, specificity, positive predictive value, and negative predictive value of sonography would be measured.

9. Recommendations for Future Research: It would be valuable to include suggestions for future research.

Response: the final paragraph of the discussion is added to serve this goal.

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 and also the inability to correlate the results of sonography with liver fibrosis as assessed by histology. For further research, prospective studies with liver biopsy evaluations are suggested so that the sensitivity, specificity, positive predictive value, and negative predictive value of sonography would be measured.

Associate Editor

Please novelty regarding the manuscript:

Response: the final paragraph of the introduction is added to the manuscript to serve this goal.

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 were shown to 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. Therefore, this study, aims to compare ultrasonographic findings with traditional liver enzyme tests for diagnosing NAFLD in both normal-weight and obese children.

We would once again appreciate your kind consideration of our manuscript. We hope that with the changes made in the manuscript, this article will be suitable for publication in your prestigious journal.

2 Revision 1

1 comment · 23 Jun 2024

Associate Editor

ACCEPT 23 Jun 2024

The manuscript has been revised according to the comments.