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OPEN PEER REVIEW

Diagnostic Performance of the Monocyte/High-Density Lipoprotein Cholesterol (HDL-C) Ratio for Pediatric Metabolic Associated Fatty Liver Disease in Children and Adolescents with Obesity

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

1 comment · 3 Mar 2024

Associate Editor

MAJOR REVISION 3 Mar 2024

Dear Author
This article is attractive and well-written. But I have some questions specifically about the case section.

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide among children and adolescents. It contains a spectrum of diseases, from its mildest form of isolated steatosis to nonalcoholic steatohepatitis (NASH) to liver fibrosis and cirrhosis, or end-stage liver disease.
Liver biopsy is the gold standard for diagnosis and staging. However, noninvasive serological biomarkers, composite scores, and imaging biomarkers are being extensively studied for the diagnosis of NAFLD, nonalcoholic steatohepatitis, and liver fibrosis in children.
Could you please describe how cases were selected and how NAFLD was diagnosed, and provide information on fibrosis, NASH, or steatosis in your study?
Furthermore, as you know, obesity and metabolic dysfunction are important clinical features of NAFLD; therefore, an international panel proposed the name 'metabolic-associated fatty liver disease' (MAFLD) to replace NAFLD in 2020 for adults. Subsequently, an age-specific definition of pediatric MAFLD was released. Compared to the definition of NAFLD, the criteria for MAFLD do not require the exclusion of other causes of steatosis, such as Wilson's disease; rather, a requirement is the presence of abnormal metabolic features, including metabolic syndrome, prediabetes, or overweight/obesity. {Eslam, M.; Alkhouri, N.; Vajro, P.; Baumann, U.; Weiss, R.; Socha, P.; Marcus, C.; Lee, W.S.; Kelly, D.; Porta, G.; et al. Defining pediatric metabolic (dysfunction)-associated fatty liver disease: An international expert consensus statement. Lancet Gastroenterol. Hepatol. 2021, 6, 864–873.}

Standard ultrasonography exhibits limited efficacy in detecting steatosis in children. Please describe the case selection for this study.

Comparative evaluation of pediatric fatty liver disease criteria: MASLD, ESPGHAN, NASPGHAN, and NAFLD – identifying the optimal pediatric label
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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.

Response to Reviewer

Reviewer 1:
Comments to the author

This retrospective study was investigate the correlation between MHR and NAFLD in children and adolescents with obesity, as well as to assess the feasibility of using MHR as a novel and convenient biomarker for the detection of NAFLD.
My comments on the manuscript can be summarized as follows:

Except for the minor comments I do not have any major comments for the manuscript for further revision/corrections.
Minor comments;
In the abstract section: “Monocyte/HDL-C ratio was significantly higher in the NAFLD group than in the non-NAFLD group (0.46±0.14 vs. 0.56±0.19, p<0.001).
Please mention the value and p value in the same order as mentioned in the text.
First, the value related to the group NAFLD group and then non-NAFLD

Reply: The text was revised following: Monocyte/HDL-C ratio was significantly higher in the NAFLD group than in the non-NAFLD group (0.56±0.19 vs. 0.46±0.14, p<0.001).

2-In different parts of the text, some sentences are not referenced, please add these items. For example, some sentences are given below. Please review the entire text in this regard

In the Background section:

In Page 5, Line 56 “The diagnosis of NAFLD is now established by a process of exclusion since there is a lack of clinically useful biomarkers available for diagnostic purposes”

Reply: Regarding your observation about unreferenced sentences in the manuscript, we appreciate your attention to detail. Upon review, we have confirmed that the statement you referenced is supported by reference [1].

(1) Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018 Jan;67(1):328–57.

In Page 5, Line 59 “Chronic inflammation is a major contributor to the development of NAFLD”

In Page 5, Line 59 “According to the Multiple Impact Hypothesis, a number of factors, including dietary factors, gut microbiota, hormones produced by adipose tissue, insulin resistance, and genetic and epigenetic factors, together contribute to the development of NAFLD by causing oxidative stress and inflammation in the liver”

Reply: Regarding your comment about unreferenced sentences in the manuscript, we appreciate your attention to detail. Upon further examination, we found that the statements you mentioned are indeed supported by reference [6] as follows:

"Chronic inflammation is a major contributor to the development of NAFLD. According to the Multiple Impact Hypothesis, a number of factors, including dietary factors, gut microbiota, hormones produced by adipose tissue, insulin resistance, and genetic and epigenetic factors, together contribute to the development of NAFLD by causing oxidative stress and inflammation in the liver (6)."

(6) Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016 Aug;65(8):1038–48.

We apologize for any confusion and oversight. We have ensured that all statements are appropriately referenced throughout the manuscript.

In Page 6, Line 71 “High-density lipoprotein cholesterol (HDL-C) exerts anti-inflammatory effects by inhibiting the pro-inflammatory and pro-oxidant actions of monocytes through the blocking of macrophage migration and LDL oxidation”

Reply: Upon further examination, we have confirmed that the statement you referenced is indeed supported by reference [10]:

"High-density lipoprotein cholesterol (HDL-C) exerts anti-inflammatory effects by inhibiting the pro-inflammatory and pro-oxidant actions of monocytes through the blocking of macrophage migration and LDL oxidation (10)."

(10) Ganjali S, Gotto AMJ, Ruscica M, Atkin SL, Butler AE, Banach M, et al. Monocyte-to-HDL-cholesterol ratio as a prognostic marker in cardiovascular diseases. J Cell Physiol. 2018 Dec;233(12):9237–46.

Reviewer 2:
This study was done with the purpose of "Diagnostic Performanc of the Monocyte/High-Density Lipoprotein Cholesterol (HDL-C) Ratio for Nonalcoholic Fatty Liver Disease in Children and Adolescents with Obesity". However, there are significant concerns that need to be addressed.
As you mentioned, in previous studies, the relationship of this index with obesity and fatty liver has been investigated, what is the reason for repeating this study again from the researchers' point of view?

Reply: Thank you for your thoughtful comments. We would like to clarify that our study does not constitute a repeate of previous research. Our study is the first to demonstrate an increased MHR (Monocyte-to-HDL Cholesterol Ratio) ratio in obese children with NAFLD compared to obese children without NAFLD. After the evaluation by the referees, we revised the article using the term "MAFLD" instead of "NAFLD" due to its inappropriate use for children. This finding is novel and contributes significantly to the existing literature on the subject.

It is important to note that previous studies, such as the one conducted by Ünal et al., have indeed investigated the relationship between MHR and obesity in children, showing an increased MHR in obese children compared to healthy non-obese children. However, our study specifically focuses on the association between MHR and NAFLD in obese children, which distinguishes it from previous research. Furthermore, while there have been studies evaluating MHR in obesity in adults, ours is among the few to explore this relationship in pediatric populations.

It seems that in the implementation method, things like the sample size and the method of patient selection should be explained in detail.

Reply: The text in the Methods section has been revised as follows: **“**The retrospective research, conducted using simple random sampling, included a cohort of individuals between the ages of 6 and 18, including children and adolescents, followed for obesity with a BMI > 95 percentile at the pediatric endocrinology clinic from October 1, 2022, to September 30, 2023.”

The standard method for comparing a new tool for diagnosing fatty liver is liver biopsy. In recent studies, it has been shown that ultrasound of the liver is not accurate enough to diagnose fatty liver, and it is better to use fibroscan even instead of biopsy, which is an invasive method, so first, justify this issue and secondly, this issue should be discussed in Limitations of the study should be clearly explained. The same is true of your claim about anti inflammatory markers.

Reply: The text in the Methods section has been revised as follows: **“**There were some limitations to our study, (1) including the use of ultrasound rather than liver biopsy or fibroscan

to diagnose hepatosteatosis. (2) As our study is a retrospective study, a judgment on the cause-and-effect relationship between MHR and MAFLD could not be reached. (3) There was no healthy control group without obesity. (4) Other biomarkers were not considered to assess inflammation. To verify the results of this study, studies are needed, compared to larger sampling groups with healthy control groups, to utilize more accurate diagnostic methods for MAFLD, and to evaluate other inflammation markers."

Are the results in the table adjusted for confounders? please explain more.

Reply: We conducted multivariate logistic regression analyses to thoroughly explore the relationship between each variable and the presence of MAFLD. Our findings revealed significant associations between certain variables and MAFLD after adjusting for potential confounders.

The following text and table were added:

We performed multivariate logistic regression analyses to investigate the association between each variable and MAFLD. There were strong links between having MAFLD and being male (OR: 3.825; 95% CI=1.673-8.740; p=0.001), having a high ALT level (OR: 1.035; 95% CI=1.004-1.070; p=0.025), and having a high MHR level (OR: 16.166; 95% CI=1.408-185.610; p=0.025) (Table 3). Considering confounding factors, MHR persisted as the most significantly associated factor with an elevated risk of MAFLD.

Tablo 3: Logistic regression analysis of factors associated with MAFLD.

Variable	B	SE	OR	95% CI	p-value
Age	0.028	0.063	1.028	0.908-1.160	0.663
Gender (1=male, 0=female)	1.341	0.421	3.825	1.673-8.740	**0.001**
BMI SDS	0.508	0.318	1.661	0.891-3.100	0.111
FBG (mg/dl)	0.016	0.025	1.016	0.969-1.070	0.505
ALT (IU/L)	0.034	0.015	1.035	1.004-1.070	**0.025**
AST (IU/L)	0.004	0013	1.075	0.978-1.030	0.786
HOMA-IR	0.072	0.057	0.989	0.962-1.200	0.202
TG/HDL	-0.011	0.267	1.365	0.587-1.670	0.968
TyG	0.311	0.959	1.004	0.208-8.940	0.745
MHR ($\times 10^9$/mmol)	2.783	1.245	16.166	1.408-185.610	**0.025**
Adjusted R2= 0.216, p=<0.001					

Table 3 Legends: Abbreviations: MAFLD, metabolic associated fatty liver disease; BMI, body mass index; SDS, standard deviation score; FBG, fasting blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HOMA-IR, homeostatic model assessment of insulin resistance; TG/HDL, **triglyceride/HDL-C ratio; TyG, triglyceride-glucose Index;** MHR, monocyte to high-density lipoprotein cholesterol ratio. β, standardized regression coefficient; SE, standard error of partial regression coefficient; OR, odds ratio; CI, confidence interval. High MHR was significantly related to risk of MALFD.

Associate Editor's Comments:

Associate Editor 1:
Dear Author
This article is attractive and well-written. But I have some questions absolutely in the case section.

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide among children and adolescents. It contains a spectrum of diseases, from its mildest form of isolated steatosis to nonalcoholic steatohepatitis (NASH) to liver fibrosis and cirrhosis, or end-stage liver disease.

Liver biopsy is the gold standard for diagnosis and staging. However, noninvasive serological biomarkers, composite scores, and imaging biomarkers are being extensively studied for the diagnosis of NAFLD, nonalcoholic steatohepatitis, and liver fibrosis in children.
Could you please mention about selection of cases and how did diagnosis of NAFLD and what about fibrosis, Nash, or steatosis in your study?
Otherwise, as you know, Obesity and metabolic dysfunction are important clinical features of NAFLD; therefore, an international panel proposed the name ‘metabolic-associated fatty liver disease’ (MAFLD) to replace NAFLD in 2020 for adults. Subsequently, an age-specific definition of pediatric MAFLD was released. Compared to the definition of NAFLD, the criteria of MAFLD do not need to exclude other causes of steatosis, such as Wilson’s disease; rather, a requirement is the presence of abnormal metabolic features, including metabolic syndrome, prediabetes, or overweight/obesity.
{ Eslam, M.; Alkhouri, N.; Vajro, P.; Baumann, U.; Weiss, R.; Socha, P.; Marcus, C.; Lee, W.S.; Kelly, D.; Porta, G.; et al. Defining pediatric metabolic (dysfunction) associated fatty liver disease: An international expert consensus statement. Lancet Gastroenterol. Hepatol. 2021, 6, 864–873.}]

Reply: Thank you for your constructive review and insightful suggestions. Your guidance has been invaluable in refining our study methodology and terminology.
In our study, we included patients who were obese with a BMI above the 95th percentile, and those who exhibited hepatosteatosis on ultrasound evaluation. In accordance with your recommendations, we determined that the term 'MAFLD' would be more appropriate. Therefore, we structured our criteria for MAFLD diagnosis to include patients who exhibited hepatosteatosis on imaging, along with at least one metabolic risk factor.
You can follow the changes throughout the text.
Once again, we sincerely appreciate your thoughtful feedback and contribution to the enhancement of our research.

Standard ultrasonography exhibits limited efficacy in detecting steatosis in children.Please describe the case selection for this study.
Comparative evaluation of pediatric fatty liver disease criteria: MASLD, ESPGHAN, NASPGHAN, and NAFLD – identifying the optimal pediatric label Published: January 13, 2024DOI:https://doi.org/10.1016/j.jhep.2024.01.004

Reply: In addressing the limited efficacy of standard ultrasonography in detecting steatosis in children, we acknowledge that ultrasonographic findings become more pronounced in the presence of hepatosteatosis exceeding 30% of liver tissue. Despite its limitations, ultrasonography remains a valuable noninvasive tool for detecting hepatosteatosis and excluding alternative underlying etiologies (5).

Furthermore, in the limitations section of our study, we acknowledged that there were some limitations, including the use of ultrasound rather than liver biopsy or fibroscan to diagnose MAFLD. This limitation is important to note, and while ultrasonography provides valuable information, it may not provide the same level of diagnostic accuracy as invasive techniques such as liver biopsy or fibroscan.

2 Revision 1

0 comments

No public comments in this round.

3

Revision 2

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.

Associate Editor 1:

Dear author, I appreciate your efforts in this article. However, to make it more robust and accurate, it would be helpful if you could consider identifying and accounting for any potential confounding variables in both the regression and correlation analysis. This will improve the overall quality of the article. Thank you.

Dear Associate Editor, Thank you for your valuable feedback and for pointing out an important aspect of our analysis that needs further attention. We have reviewed our data to identify potential confounders that could affect the relationship between our variables of interest.

The multivariate logistic regression includes age, gender, BMI SDS, fasting blood glucose (FBG), ALT, AST, HOMA-IR, TG/HDL, and TyG as covariates, alongside MHR.

Correlation analysis performed between the MHR and age, weight SDS, BMI SDS, fasting blood glucose (FBG), ALT, AST, fasting insulin, HbA1c, HOMA-IR, TG/HDL, TyG, total cholesterol, triglyceride, LDL-C and grade of hepatosteatosis.

We have updated the legends for the relevant tables to clearly state the inclusion of confounding variables in the analysis, as well as to explain the rationale behind the specific variables chosen for adjustment.

We are grateful for your constructive comments, which have helped us strengthen the scientific rigor of our work.

Reviewer 1:

The requested items have been applied in the text by the authors.

Dear Reviewer,

We appreciate your careful review and constructive comments, which helped us improve the clarity and rigor of our manuscript.

Reviewer 2:

The study with title "Diagnostic Performance of the Monocyte/High-Density Lipoprotein Cholesterol (HDL-C) Ratio for Pediatric Metabolic Associated Fatty Liver Disease in Children and Adolescents with Obesity" is very accurate, however, there are several details that should be noted.

Dear Reviewer,

We appreciate your recognition of the study's accuracy, as well as your suggestion to address additional details for a more comprehensive understanding of our findings.

1. In the limitations of your study, how did you conclude that future studies should be conducted with a larger sample size? Wasn't the sample size of this study measured in principle? And based on what variable? please mention.

Thank you for your comments and suggestions. We acknowledge that the sample size in our study was determined through simple random sampling within a retrospective study framework. Because our study is retrospective, the data collection process was completed in the past and was limited to the available data scope. By using simple random sampling, we aimed to create an unbiased sample from the existing data set. However, this method can sometimes lead to insufficient representation of specific subgroups or limitations in statistical power due to a smaller sample size. The reason we suggest larger sample sizes for future studies is to overcome these limitations. Larger samples offer the potential for greater representation across various subgroups, and they can increase the statistical power of analyses, thereby providing more robust and reliable results.

2. You did not mention confounding variables in the regression and correlation analyses. Since this study is a retrospective study, it is not possible to adjust these variables during design, and the only way is to adjust these variables in statistical analyses. Therefore, write them in the caption of the tables.

We added following text in the legend of the table 2: “Correlation analysis performed between the MHR and age, weight SDS, BMI SDS, fasting blood glucose (FBG), ALT, AST, fasting insulin, HbA1c, HOMA-IR, TG/HDL, TyG, total cholesterol, triglyceride, LDL-C and grade of hepatosteatosis. MHR.”

We added following text in the legend of the table 3: “The model adjusted was for age, gender, BMI SDS, FBG, ALT, AST, HOMA-IR, TG/HDL, and TyG.”

3- In the discussion of the study, pay more attention to past studies in this field and whether measuring such a variable can be cost-effective in your opinion and based on other studies?

We added following text in discussion section: “As reported in previous studies, the MHR is an easy, cost-effective, and predictive marker for identifying MAFLD in children and adolescents.”

3 Revision 2

0 comments

No public comments in this round.