Peer Review File

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Reviewer A:

In the present article, the authors have investigated a possible association of the ratio of γ-glutamyl transpeptidase to high-density lipoprotein cholesterol (GGT/HDL) and prevalence of NAFLD or MS. In addition, the same ratio was tested as possible predictive factor for NAFLD. The results demonstrated that as GGT/HDL-C ratio increased, the prevalence of NAFLD and HOMA-IR gradually increase. In addition, the results demonstrated that GGT/HDL-C is a predictive factor for NAFLD.

To my view, the authors have not clearly distinguished between the association of the ratio GGT/HDL with the prevalence of NAFLD or MS with the prediction factor for NAFLD. Thus, I recommend the authors emphasize and clarify this difference in the title, abstract, results and conclusions.

Response: We have analyzed the GGT/HDL-C ratio, performed the statistical analyses again, revised the Title, Abstract, Results, and Conclusions sections, and clarified the association of GGT/HDL ratio with prevalence of NAFLD/MS.

Secondly, a real limitation of the study is based on the fact that the two groups of patients were dramatically different. How could the authors be sure to have properly correct to all these variables?

Response: Our study involved a consecutive retrospective cohort between July 2014 and November 2017 with a sample size of 6326 patients after applying the exclusion criteria. Because we wanted to examine the prevalence of NAFLD/MS in the Results section, we did not use a matched non-NAFLD group. In the regression analyses, we have corrected the predicted value of the GGT/HDL-C ratio for NAFLD/MS by adjusting for baseline indexes and multiple variables. However, the two groups of patients were dramatically different in their baseline characteristics, which was a limitation of the study. Therefore, we have included this point in the limitations section of the revised manuscript. (page8, lines 305-306)

Reviewer B:

This is an intriguing study. The association of elevated levels of GGTP with liver disease has been demonstrated but the association of higher normal levels of GGTP in association with NAFLD is relatively new. In addition, the protective effect of HDL-C on cardiovascular health has also been demonstrated, but the association with NAFLD is also relatively recent. The novel finding of this paper is that the ratio of GGTP/HDL-C is more accurate in assessing risk for NAFLD than either GGTP or HDL-C alone. While there are limitations of this paper, which are stated in the Discussion, this will provide a nice background for others to validate this as a useful marker for NALFD risk.

Queries:

1) were patients' medications analyzed? Specifically, drugs known to increase HDL-C, such as niacin; fibrates such as gemfibrozil (Lopid); and certain statins, particularly simvastatin (Zocor)

and rosuvastatin (Crestor).
2) In Tables 1 and 2, it is unclear what the values are that are in parenthesis.
Response: We analyzed the patients' medications. The personal history for the patients included alcohol consumption, history of liver disease, hypertension, and diabetes, and medication use for hypertension, hyperlipidemia, and diabetes. We have corrected the values in parentheses shown in the tables.

Reviewer C:

The manuscript is very interesting and it can add additional new findings about novel and non-invasive predictors for liver damage in NAFLD. There are a lot of unnecessarily results which draw the reader attention away from the main findings. The manuscript can be greatly improved after the comprehensive and major corrections.

MAJOR COMMENTS
Background
This section needs to be rewritten in the context to explain roles and causality of GGT and HDL-C as single markers in liver damage and progression from simple steatosis to steatohepatis. The authors stated results of different published papers indicating only associations between GGT and other liver enzymes with NAFLD. If possible explain in one sentence why the prognostic value of single GGT and single HDL-C measurements is limited. The objective of the study (…to investigate the predictive value of GGT/HDL-C ratio for NAFLD and to evaluate the diagnostic efficacy of GGT/HDL-C ratio in NAFLD in a Chinese general population) was not supported by the results because the authors used quartile values and not row ratio values for NAFLD prediction and diagnostic efficacy (Tables 4 and 5). This needs to be corrected in order to have the manuscript published.
Response: We have revised the Background section to indicate that single increase in GGT can be used as an indicator of steatosis in liver cells, and that single decrease in HDL-C is associated with IR and dyslipidemia, based on previous studies. However, the prognostic value of single GGT and single HDL-C measurements is limited. Given that GGT and HDL-C are both associated with NAFLD, we calculated their ratio, and speculated that GGT/HDL-C ratio may combine both functions to indicate NAFLD. We have analyzed the GGT/HDL-C ratio, performed the statistical analyses again, revised the Title, Abstract, Results, and Conclusions sections, and clarified the association of GGT/HDL ratio with prevalence of NAFLD/MS.

Methods
The authors included 1813 participants with NAFLD. How many of them had NASH? Please include this data in the participant description.
Response: Ultrasonography is the most inexpensive and widely available imaging test for NAFLD. Liver biopsy is the gold standard for diagnosis of NAFLD/NASH, but has inherent limitations because of high cost, bleeding risk, sample size, and sampling variability. Liver biopsy to diagnose NASH or fibrosis would be an enormous task and impractical for routine use. Therefore, it is important to identify and validate non-invasive markers for predicting NAFLD/NASH. Kobayashi et al. (reference 1) indicated that FIB-4 index (FIB-4 >1.56) had good diagnostic ability for NASH. We identified 252 (13.9%) NASH patients among 1813

NAFLD patients using FIB-4 >1.56. However, because we did not specify NASH patients in our study, we cannot list the specific number of NASH patients.

Are there any participants with type 1 diabetes? Did You include them in the study or not?
Response: There were no patients with type 1 diabetes in our study. Substantial evidence has indicated that hepatic steatosis is associated with type 1 diabetes complications of retinopathy, cardiovascular disease, and polyneuropathy. Thus, we did not deliberately exclude patients with type 1 diabetes, but did not find any such patients among our 6326 participants (references 2-4, the studies of Alessandro, Tripolino, and Giovanni et al.).

Citation 16 is not good for citation for MS diagnostic criteria. Please, include appropriate reference for MS diagnostic criteria.
Response: We have updated the citation for MS diagnostic criteria in the revised manuscript, and cited the report shown below.
Weng J, Ji L, Jia W, et al. Standards of care for type 2 diabetes in China. Diabetes Metab Res Rev 2019;32:442-58.

Why the authors included all of these biomarkers: ALT, AST, alkaline phosphatase (ALP), TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), GGT, FPG, creatinine (Cr), uric acid (UA), total protein (TP), albumin (ALB), total bilirubin (TB), insulin (INS), hemoglobin (Hgb), and platelet count (PLT)) when most of the were not further discussed in the context of fatty liver disease in the discussion section? It is essential to give short explanation about them or to remove some of them from the manuscript.
Response: We have deleted the data for total protein (TP), albumin (ALB), total bilirubin (TB), hemoglobin (Hgb), and platelet count (PLT). We used fasting insulin (INS) and glucose (Glu) for calculation of homeostasis model assessment of insulin resistance (HOMA-IR), and used γ-glutamyl transpeptidase (GGT) and high-density lipoprotein cholesterol (HDL-C) for calculation of GGT/HDL-C ratio. In the revised manuscript, we have described and discussed the results for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), creatinine (Cr), and uric acid (UA) in the Results and Discussion sections.

Statistical analysis
This section needs to be comprehensively revised.
How the authors tested data distribution?
Please use median and interquartile range and not the range.
What test was used for comparison of categorical data?
Please explain why multinomial logistic regression analysis was used and not binary logistic when there are two groups of examinees NAFLD vs non-NAFLD. Also, the authors stated "Multinomial logistic regression was used to evaluate the association between GGT/HDL-C ratio and NAFLD" but in tables they used quartile values.
The authors did not mention how data from logistic regression and ROC analyses were presented within results.
Response: We have revised the statistical methods. One-sample Kolmogorov–Smirnov tests were used to assess the normality of data distributions. Data were presented as mean ± standard

deviation when the distribution was normal and median (interquartile range) when the distribution was skewed. The chi-square test was used for comparisons of categorical data. Binary logistic regression was used to evaluate the association between GGT/HDL-C ratio and NAFLD. The quartile values of GGT/HDL-C ratio were changed to continuous GGT/HDL-C ratio, and the relevant results were re-analyzed to evaluate the association between GGT/HDL-C ratio and NAFLD (red font in second part of Results section). The logistic regression and ROC results have been explained in detail. (page4-5, lines 143-161)

Results

The results section must be significantly shortened and are needed to support the conclusions of the manuscript.

Baseline characteristics of the participants:

Table 1: There are a lot of mistakes in this table:

1. Categorical data are given as absolute and relative frequencies and in the statistical analysis is written differently.
2. HDL-c or HDL-C. LDL-ch?
3. There are no explanations about data below the table. Must include how data are presented and with what tests were they compared.
4. How many of them were with type 2 diabetes?
5. Table is too big, remove useless biochemical markers which does not support the conclusions.

Response: We have corrected the mistakes in Table 1.

1. The descriptions of categorical data have been made consistent between the statistical analysis section and Table 1.
2. The abbreviations of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol have been unified as HDL-C and LDL-C, respectively.
3. We have explained the data representation and relevant statistical methods in the footnotes to Table 1.
4. The number of people with type 2 diabetes has been added to Table 1.
5. We have deleted the data for total protein (TP), albumin (ALB), total bilirubin (TB), hemoglobin (Hgb), and platelet count (PLT), and simplified Table 1.

Characteristics of NAFLD participants according to quartiles of GGT/HDL-C ratio:

Why participants were divided in quartiles and not tertiles (Table 2)? Table 2 needs to be removed because it does not give any additional information towards the manuscript conclusions. It is also too big. It has the same omissions like Table 1. Also, it cannot be seen from the Table 2 between which quartile groups differences existed. This is obligatory to be indicated in the table?

Response: We have deleted the original Table 2.

Figure 1:

1. Why did the authors include MS in the Figure 1? If including MS within this figure and Tables 3, 4 and 5, the manuscript title should be changed to “Association between ratio of γ glutamyl transpeptidase to high density 1 lipoprotein cholesterol and prevalence of nonalcoholic fatty liver disease and metabolic syndrome: a cross sectional study”.

2. What statistical test was used to test percentages given in this section (from 161 to 167 lines)? This was not mentioned in the statistical analysis.
3. Why were the patients further classified in underweight, normal weight, overweight and obese within this figure?
4. The same as for the Table 2, I recommend Figure 1 to be excluded from the present manuscript.
Response: We appreciate the reviewer's suggestions. We have deleted the original Figure 1 and briefly described the prevalence of MS and NAFLD by GGT/HDL-C quartiles in the Results section. Because NAFLD is strongly associated with MS, we have changed the title to "Association between ratio of γ-glutamyl transpeptidase to high-density lipoprotein cholesterol and prevalence of nonalcoholic fatty liver disease and metabolic syndrome: a cross-sectional study". Comparisons of the prevalence of MS and NAFLD by GGT/HDL-C quartiles were carried out by the Cochran–Armitage trend test. While NAFLD was strongly associated with obesity and MS, a proportion of NAFLD patients had relatively normal BMI. We wanted to examine whether GGT/HFL-C ratio changed in different body weight groups.

Correlation analyses between GGT/HDL-C ratio and other variables:
1. Why authors used r and not ρ for Spearman correlation analysis (lines 171-174)?
2. What does "closely associated" (line 175) mean when all correlations were significant at level $P<0.001$?
3. Is it possible that $r=0.071$ (line 172) had significance of $P<0.001$? Please, check this again in the statistical programme.
Response: We have used "P-values" for Spearman correlation analyses and corrected the incorrect expressions. (page5, lines 187-190)

Association between GGT/HDL-C ratio and prevalence of NAFLD or MS:
Table 3:
1. Exclude quartiles and put the row data GGT/HDL-C in this table and calculated the odds. Beside the above mentioned reasons for removing quartiles, odds for forth quartile for both NAFLD and MS are unacceptable.
2. Why did the association with NAFLD and MS are tested for all biochemical markers?
3. What is Tch?
Response: We have analyzed the GGT/HDL-C ratio, deleted the data for total protein (TP), albumin (ALB), total bilirubin (TB), hemoglobin (Hgb), and platelet count (PLT), and left the following disease-related markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), homeostasis model assessment of insulin resistance (HOMA-IR), and uric acid (UA). We then performed the statistical analyses again. Tch indicated total cholesterol (TC), and we have corrected this abbreviation. (page6, lines 201-204)

Table 4:
1. Exclude the quartiles and use row data for GGT/HDL-C. Perform binary logistic using independent variable 0-noNAFLD and 1-NAFLD.
2. Why did authors create 6 models and how did they selected biomarkers to get into the models?

Response: We have analyzed the GGT/HDL-C ratio and performed binary logistic regression using independent variables of 0 for non-NAFLD and 1 for NAFLD. We used age, WC, BMI, SBP, DBP, sex, and smoking as baseline indexes, ALP, ALT, and AST as liver enzyme biomarkers, Cr and UA as kidney biomarkers, TC, TG, and LDL-C as lipid markers, and HOMA-IR as a glycometabolism marker. In the revised manuscript, we have created five models and made adjustments for age, WC, BMI, SBP, DBP, sex, smoking, ALP, ALT, AST, Cr, UA, and HOMA-IR in multivariate analyses to correct for the effects of these markers on the application value of GGT/HDL-C ratio. We initially investigated whether this ratio was valuable for NAFLD after adjustment for baseline indexes (model 1), and subsequently continued to add liver, kidney, lipid, and glycometabolism markers in turn to investigate whether the ratio was valuable for NAFLD after adjustment for baseline indexes, and liver, kidney, lipid, and glycometabolism markers. (page6, lines 202-207)

Table 5:
1. Exclude the quartiles and use row data for GGT/HDL-C. Perform binary logistic using independent variable 0-noNAFLD and 1-NAFLD.
2. Why did authors classified patients in normal weight and overweight and further statistically tested? Why omitted obese ones?
Response: We have analyzed the GGT/HDL-C ratio and performed binary logistic regression using independent variables of 0 for non-NAFLD and 1 for NAFLD (shown in Table 4). Because the original Table 2 was deleted, the original Tables 4 and 5 have been renamed Tables 3 and 4, respectively. We want to examine the predictive value of GGT/HDL-C ratio for NAFLD in different body weight groups. Therefore, Table 3 shows all included subjects (6326), while Table 4 shows subgroup analyses for different body weight groups based on Table 3. Because the number of people with low body weight was too small and there was no NAFLD population (total number 152, NAFLD 0), no statistical analyses were performed. The majority of obese people were NAFLD patients (total number 693, NAFLD 515), and this group was also not suitable for regression analysis. (page6, lines 208-221)

Predictive value of GGT/HDL-C ratio for prevalence of NAFLD:
What statistical test was used to test the differences between areas of ROCs (lines 204 and 205)? Insert AUC values for all 3 ROC in the figure 2?
Response: The Z statistical test was used to test the differences between areas under the ROC curves. This was done manually or by the Delong method using MedCalc software. We have inserted the AUC values for all three ROC curves in the revised figure. The manual calculation formula is shown below. (page5, lines 157-161)
$Z = |AUC1–AUC2|/SQRT[(SE1)^2+(SE2)^2]$
P = [1-NORMSDIST(Z value)]×2

Discussion
Lines 249-263 – better explanations about the role of HDL-C (and not TG/HDL-C) is needed in the context of NAFLD. The authors did not tested GGT/TG as a predictor for NAFLD.
Lines 264-267 – Based on what results the authors made such a conclusion. They did not test associations of GGT/HDL-C with oxidative stress and systemic inflammation in NAFLD patients.

Please explain in the discussion section whether or not AUC, specificity and sensitivity of GGT/HDL-C have any clinical significance.

Response: We have revised the relevant contents and explained that AUC, specificity, and sensitivity of GGT/HDL-C ratio had some clinical significance in the Discussion section. (page7-8, lines 272-298)

References:

1. Kobayashi N, Kumada T, Toyoda H, Tada T, Ito T, Kage M, Okanoue T, Kudo M. Ability of Cytokeratin-18 Fragments and FIB-4 Index to Diagnose Overall and Mild Fibrosis Nonalcoholic Steatohepatitis in Japanese Nonalcoholic Fatty Liver Disease Patients. Dig Dis. 2017;35(6):521-530. doi: 10.1159/000480142. Epub 2017 Oct 17.

2. Alessandro Mantovani, Riccardo Rigolon, Lucia Mingolla, Isabella Pichiri, Valentina Cavalieri , Laura Salvotelli, Vincenzo Stoico, Giacomo Zoppini, Enzo Bonora, Giovanni Targher. Nonalcoholic Fatty Liver Disease Is Associated With an Increased Prevalence of Distal Symmetric Polyneuropathy in Adult Patients With Type 1 Diabetes. J Diabetes Complications, 31 (6), 1021-1026

3. Tripolino C, Irace C, Cutruzzolà A, Parise M, Barone M, Scicchitano C, Cortese C3, Gnasso A. Hepatic Steatosis Index Is Associated with Type 1 Diabetes Complications. Diabetes Metab Syndr Obes. 2019 Nov 20;12:2405-2410. doi: 10.2147/DMSO.S221969. eCollection 2019.

4. Giovanni Targher, Amedeo Lonardo, Christopher D Byrne. Nonalcoholic Fatty Liver Disease and Chronic Vascular Complications of Diabetes Mellitus. Nat Rev Endocrinol Actions 14 (2), 99-114 Feb 2018.