Author’s response to reviews

Title: Low Vitamin D Level Was Associated With Metabolic Syndrome And High Leptin Level In Subjects With Nonalcoholic Fatty Liver Disease: A Community-based Study

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Author’s response to reviews:

Dear Professor Anna Melidoni and Guangde Tu, editor, BMC Gastroenterology

Thank you very much for your constructive comments on our manuscript. My colleagues and I greatly appreciate the suggestions offered by you and the reviewers, and the opportunity to improve our manuscript. We have made the following changes according to the suggestions of you and reviewers:

Technical Comments:

1. Please reformat and rephrase parts of the Declarations sections in accordance to our submission guidelines

https://bmcgastroenterol.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article

Reply: We have reformatted and rephrased parts of the Declarations sections in accordance to BMC Gastroenterology submission guidelines.
2. Please confirm whether informed consent, written or verbal, was obtained from all participants in the “Ethics approval and consent to participate” section of the Declarations. If verbal, please state the reason and whether the ethics committee approved this procedure.

Reply: All the participants agreed to attend the study and provided “written” informed consent before enrollment in this study. The type of informed consent has been added in the section of “Material and methods” (Page 6, line 100). We also add the informed consent type in the revised “Ethics approval and consent to participate” section (Page 20, line 351).

Reviewer 2, Mandana Rafeey: accepted

Reviewer 3, Anam Khan, has four questions:

Q1: The manuscript has a few limitations. The authors use abdominal ultrasound to determine the severity of hepatic steatosis. On page 12, line 206-208, the authors state "The degree of fatty liver disease was graded as normal (absent), mild, moderate or severe, on the basis of the intense reflection level of echogenicity". Liver biopsy is currently the most reliable approach for identifying for the presence of steatohepatitis and fibrosis in patients with NAFLD. Imaging tests such as ultrasound do not reliably reflect the spectrum of liver histology in patients with NAFLD. Imaging such as with magnetic resonance elastography or transient elastography are validated noninvasive imaging modalities for assessment of advanced fibrosis in patients with NAFLD which were not used by the study authors.

Reply: Thank you for the important comments. We agree that liver biopsy is currently the most reliable approach for identifying for the presence of steatohepatitis and fibrosis in patients with NAFLD. In this study, liver biopsy could not be performed in the community mass screening study. Abdominal US was applied to assess the severity of steatosis and fibrosis. Although two operators who were blinded to the laboratory data performed abdominal US, the results of abdominal US findings are relatively subjective. We also agree that applying a validated noninvasive method, such as transient elastography (Fibroscan), to assess the steatosis and fibrosis in patients with NAFLD is warranted in the further studies.
Q2: The authors do calculate the NAFLD fibrosis score which is a validated clinical decision aid to predict likelihood of advanced fibrosis. However according to the authors, most subjects with NAFLD did not have an advanced fibrosis status. On page 13, line 281, the authors state “77% of subjects present a NAFLD fibrosis score < -1.455, indicating a low degree of fibrosis (F0-2). Only 6 subjects with a NAFLD fibrosis score > 0.674 (high degree of fibrosis, F3-4) were identified in this study”. Hence, the authors could not draw any conclusions regarding an association between vitamin D levels and fibrosis in subjects with NAFLD. The authors should include that one of the limitations of their study was the inclusion of a majority of patients with NAFLD without advanced fibrosis based on NAFLD fibrosis scores. This could explain why no association was found between serum vitamin D levels and NAFLD.

Reply: We appreciate the valuable comments. But the status of steatosis and necroinflammatory reactions may disappear in patients with NASH during advanced fibrosis or cirrhosis, and this status is known as “burn-out NASH” (Takahashi Y, WJG 2014; Tiniakos DG, Eur J Gastroenterol Hepatol 2010; Caldwell SH, Ann Hepatol 2009 ). In our study, advanced fibrosis (score > 0.674) by NAFLD fibrosis score could be detected in both NAFLD group and normal group. We could not identify whether the subjects with advanced fibrosis in normal group are burn-out NASH or not. Moreover, the prevalence of advanced fibrosis was low in our NAFLD subjects, which was similar with other Asia reports (Vincent Wai-Sun Wong, Am J Gastroenterol 2008; Wai-Kay Seto, J Gastroenterol 2017). Because of low rate of patients with NAFLD and advanced liver fibrosis in present study, we could not draw any conclusions regarding an association between vitamin D levels and fibrosis in subjects with NAFLD.

Q3: Hence, this reviewer recommends that the association of vitamin D with severity of NAFLD be removed from the primary aim if using abdominal ultrasound alone to determine severity of NAFLD. The primary aim should be limited to determine the association of vitamin D with NAFLD.

Reply: We appreciate and agree with the important comments. We revised the primary aim and removed the association of vitamin D with severity of NAFLD as reviewer’s suggestion. The revised primary aim was to determine the association of vitamin D with NAFLD. We explain why the associations between vitamin D level and the severity of hepatic steatosis or fibrosis could not be analysed in the limitation section.
Q4: The authors have mentioned that one of the strengths of their study was that they measured the serum levels of inflammatory cytokines e.g. adiponectin, leptin, CRP and TNF-alpha. The secondary aim of the study should be explicitly stated to reflect that the association between serum vitamin D levels and levels of adiponectin, leptin, CRP and TNF-alpha was to be determined.

Reply: Again, we appreciate and agree with the important comments. We revised the secondary aim to address the association between serum vitamin D levels and levels of adiponectin, leptin, CRP and TNF-alpha. In bivariate correlation study, vitamin D level was not associated with TNF-α, HS-CRP or adiponectin levels. But vitamin D level was negatively correlated with leptin. Previous studies have reported vitamin D could mediate the inhibition of leptin (Hajimohammadi M et al, Eur J Clin Nutr 2017). Another study reported increased leptin level was linked to decreased HDL-C level (Rainwater DL et al, Atherosclerosis 1997). In current study, subjects with vitamin D deficiency and NAFLD had increased leptin value but lower HDL-C level. It could partially explain the association between low vitamin D level and MS in subjects with NAFLD. A further study of the potential associations among vitamin D, leptin, MS and NAFLD is warranted (Eliades M et al, World J Gastroenterol 2015; Kwok RM et al, Hepatology 2013; Skaaby T et al, Endocrine 2014) (Page 15-16, line 274-282).

Enclosed please find our revised manuscript. I hope that it is now acceptable for publication in BMC Gastroenteriology.

Thank you very much indeed for your kind attention, patience and help.

Looking forward to hearing from you at your earlier convenience.

Best Regards,

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