Author's response to reviews

Title: Prevalence of Non-Alcoholic Fatty Pancreas Disease (NAFPD) and Its Risk Factors among Adult Medical Check-Up Patients in a Private Hospital: A Large Cross Sectional Study

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Author's response to reviews: see over
1. The prevalence of NAFPD was ever reported to be around 12~16% via abdominal ultrasound, abdominal MRI or EUS in Korea, Taiwan and Hong Kong in recent years. However, it is really difficult to estimate the prevalence of NAFPD in general population due to the limitation of detecting technique and cost for measuring fatty pancreas. Therefore, the subjects receiving medical check-up seemed to be one reliable population to estimate the prevalence. However, this estimation usually will be over-estimate because such subjects may belong to high social economic groups in certain areas and the authors had raised such discussion. The report in Hong Kong was carried out on 685 healthy volunteers from the general population, with average aged around 52 in subjects of fatty pancreas. The report in Taiwan was carried out in 8097 health check-up subjects, with average aged around 56 in subjects of fatty pancreas. In this report, the studied subjects were around 43 years old, younger compared with other reports. Such difference should be mentioned in discussion. (This issue has been already added / explained in the discussion).

2. On the other hands, this study shared the same trend of NAFPD in comparison with prior reports in male gender, middle aged man, and parameters of metabolic syndrome to be the independent risk factors in subjects with fatty pancreas. (This comment is noted).

3. The results of diabetes mellitus fail to show significant meaning in multivariate analysis may be due the small sample size in subgroup statistics. This point should be point out in discussion. (This matter is already added in the manuscript).

4. The abdominal ultrasound was a cheaper, convenient and reproducible technique in detecting fatty pancreas in comparison with MRI. But ultrasound was a qualified technique and MRI belonged to be quantified. MRI, expensive technique, could indicate more accurate evaluation in fatty pancreas. Such points was suggested to be discussed in discussion. (This matter is already added in the manuscript).

5. In real world, there were still very few reports in NAFPD in different countries. The difference of fatty pancreas in various countries, races are still to be enigmatic. Such reports of prevalence from big data analysis should be encouraged to explore the real face of ectopic fatty infiltration in pancreas. (This comment is noted).

Major points:
1. The discussion about the different radiologic modalities in determining the intrapancreatic fat content is completely missing, despite that these days likely more accurate radiologic diagnostics became available such as the MRI/MRS based methods (Dixon’s two point technique corrected by the magnetic resonance spectroscopy – e.g.: Sijens et al. World Journal of Gastroenterology 2010;16:1993-1998.) and also the EUS (that is obviously less feasible for such
purpose due to its invasive nature). In contrast authors cite a reference which is more than 30 years old (from 1980) by Marks et al. when describing the method they used. I suggest to briefly compare the methods in the introduction section with a reasoning that why they have selected the US based diagnosis (in comparison with the more accurate MRI/MRS based methods). Also please update the literature referenced for US to a more recent review. (The literature has been updated and discussed more about the use of abdominal ultrasound).

2. In the view that BMI is a metabolic risk factor, but also a confounding factor in ultrasound based visualization of the pancreas (in line with abdominal circumference) – I suggest to implement a separate Limitations section where they discuss that one of their most important outcome result as a risk for having NAFPD is also a confounding factor of the method they used for the diagnosis of NAFPD. In the limitation section point 1. may also be included. (This matter is already added).

3. Did they measure abdominal circumference or BMI only? If there is no data please include a reasoning why. (The reason has been added based on our MCU standard examination).

4. In table 2 – it is somewhat unexpected to have the cholesterol levels (total, LDL, low HDL) to be so significant and pose such a high OR for having NAFPD – also very unusual and highly unexpected for systolic blood pressure that poses a higher OR for having NAFPD than diabetes mellitus itself – so please re-check data and if no change is OR and p-values discuss it extensively (i.e. why systolic bp is a stronger risk factor for NAFPD than DM). (The OR of DM and other variables mostly around the value of 2.0; so although systolic BP have higher OR, the difference with DM or triglycerides, for instance, is not large. However, the number of subjects with DM is relatively small compared to other variables. It could affect the statistical calculation of OR and may explain the slight difference with systolic BP. From the statistical point of view, clinical value of an OR occurs when it is more than 1.5. Compared to age or BMI (which have OR >4.0), the ORs of DM and systolic BP are much weaker, it’s also the same with other variables with OR value less than 2.0. From the metabolic point of view, DM is considered as part of metabolic syndrome with could confound other variables within the metabolic markers group (such as cholesterol or TG). On the other hand, blood pressure is a hemodynamic marker. High blood pressure in metabolic syndrome is a consequence of insulin resistance with various underlying mechanisms.* Therefore, higher OR of systolic BP might reflect that is more important as risk factor than DM or other lipid parameters.)

5. Were routine liver tests done? Specifically a relation of NAFPD to AST/ALT ratio or to gGT levels would be expected (possibly stronger than that of with systolic and diastolic blood pressure). If there were no liver test measures please indicate why. (Routine liver function test was done, but not included in the table because majority of NAFPD patients still have normal AST/ALT ratio. So, it seems that liver enzymes are not relevant as risk factors of NAFPD.)

6. Despite that 62 patients with diabetes mellitus (I assume type 2, but please clearly indicate it) participated in the study there is a complete lack of data on their drug use. Specifically PPAR\(\gamma\) agonist, statin, DPP-4 inhibitor and GLP-1 analouge/mimetic drug use and its relation to NAFPD would be interesting (and possibly metformin also, yet we do know that metformin fails to upregulate adipokine hormone levels therefore not specifically beneficial in NAFLD). So please include drug use in the analysis. (This study is not aimed to see the medication as independent risk factor but more in the metabolic factors.)

7. How was the lack of having diabetes mellitus diagnosed? 75g carbohydrate OGTT, normal HbA1c level? if none of these, and it was based only on medical history and fasting glucose levels, than this should be discussed in the limitations section, as in this age group a single fasting glucose level may not always be sufficient to exclude diabetes mellitus or IGT and this has an impact when authors calculate OR of DM for NAFPD. (This is MCU setting as our hospital MCU standard examination to diagnose DM).

8. Were there any data on HbA1c levels in the group of patients with DM? If so please do a calculation if the mean of the last 3-5 HbA1c levels do correlate with the presence of NAFPD. (N/A).

9. Data presentation is misleading in table 2, in terms that when authors indicate relative proportions (as %) it should be calculated otherwise: i.e. if they do have 31 patients with T2DM in the NAFPD group of 315 individuals and they have 31 patients with T2DM in the normal pancreas group of 586 individuals the relative proportion should not be 50-50% as they currently indicate - rather the relative prevalence of T2DM should be: 9.8% in the NAFPD group in contrast to 5.3% among patients who were with normal US pancreas morphology. Please do correct the relative proportions accordingly for all 11 (or if extended with liver tests than for all) variables assessed. (The data has been already changed).

10. Authors do make substantial conclusions – even in the abstract – about NAFPD’s role in malignancy, despite they do not have any cancer or cancer related outcome measure. I agree with mentioning this in the discussion section and refer to results by other study groups as pancreatic malignancy is still posing an unresolved problem and its relation to diabetes mellitus is well established, yet authors are supposed to make conclusion about their own observations – i.e. without any cancer or cancer related outcome I would remove it from the
conclusion and much more moderately mention it in the discussion section in the view of the results by others. (Well noted).

11. Isn't Beta-cell lipotoxicity and subsequent Beta-cell dysfunction important from the point of diabetes mellitus when there is an excessive ectopic fat deposition also in the pancreas? Why do authors only mention insulin resistance, when the association between diabetes mellitus and NAFPD is discussed? (Because it is the available predominant theory).

Minor comments:
1. How was the fasting glucose levels measured? Capillary blood glucose level or venous plasma? if the latter please use the term FPG (fasting plasma glucose level) instead of FBG. (It has been changed).

2. Any further explanation for why their results on NAFPD prevalence (35%) deviate so significantly from the regional Taiwanese study (US based diagnosis with more than 8000! participants – Ou et al. Plos One 2013 May 3) and from the Hong Kong study (an MRS based diagnosis in 685 individuals – Wong et al. Gastroenterology 2014;109:589-97) as both measured a 16% NAFPD prevalence? Please discuss this difference more extensively. (This issue has been discussed).