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Responses to the Editor

Manuscript Ref Number: 68392

Manuscript Title (old): Microbiota-Associated Fatty Liver Diseases

(New) Significance of Gut Microbiota in Alcoholic and Non-Alcoholic Fatty Liver Diseases

Manuscript Submission Category: Frontier

Journal: World Journal of Gastroenterology

Subject: Response to reviewer comments and suggestions of manuscript of 68392

Corresponding Author: Dong Joon Kim

Dear Editor in Chief

We thank to all reviewers and the section editor for their revision and thoughtful comments on our manuscript. We have carefully taken their comments into consideration in preparing our revision for publication, which has resulted in a paper that is clearer, more compelling, and broader. The following summarizes how we responded to reviewer.

We thank you for your time and look forward to your reply.

Sincerely Yours,

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Point by point reply of reviewer's comment

At first, we would like to express our immense regards and gratitude to both the reviewers for their precious time investment in the process of the manuscript reviewing which helped us to improvise this manuscript.

Reviewer 1:

In the manuscript entitled "Microbiota-Associated Fatty Liver Diseases" the author reviewed the significance of gut microbiota in the context of AFLD and NAFLD. Since these two liver diseases are rising sharply and the exact etiologies (particularly for NAFLD) are poorly understood reviews like this should be added to the growing body of literature in this field. I have several minor comments, which I would request the author to address and modify the manuscript accordingly.

Reply: On the behalf of my team**,** I convey my best gratitude to the Reviewer1 for his/her comments, which helped us to express our thought in conceptual way and assisted us to improvise this manuscript relativity to the topic and readability to the general population.

Comment 1: The most important point is that the author needs to explain the healthy gut microbiota citing the state of the art literature. Differences in gut microbiota according to geographical variations may also be mentioned. This should be done using a separate heading right after the introduction and before discussing the dysbiosis and the possible role of microbes in the liver diseases.

Reply: We are very thankful to the reviewer for this valuable and reasonable comment. As per your suggestion, we have added the healthy gut microbiota related information in a new section which is titled as "GUT MICROBIAL COMMUNITY EUBIOSIS" after the introduction and before discussing the dysbiosis and the possible role of microbes in the liver diseases. This new section starts from line 163 to 198. It also included their references. Included segment is below

" The gut microbiota is an endogenous ecosystem which coevolved with the host as a symbiotic organ and regulates the normal physiological functions of the gut like digestion of food, nutrients absorption and provide essential micronutrients to the host[31]. The gut microbial ecosystem maintained a balanced between the microbial species living inside the gut known as "eubiosis" and crucial for good health. The microbial colonization in the gastrointestinal tract starts immediately after birth dominated by *Bifidobacterium* genus and decline in this dominance observed in the first year of infancy[32]. The infant gut microbiota is changeable as this microbial colonization affected by multiple external factors such as mode of delivery, medications, nourishment[33, 34], age, genetic background, and cultural/geographic influence[32, 35] [36]. Like, breast-fed infants has less divers gut microbiota compared to the formula-fed infants which is the best possible explanation for the difference between US infants gut microbial composition compared to non-US infants, as they have 28 Operational Taxonomical Units (OUT's) dominated by *Prevotella* genus[32]. As children's start taking solid foods the gut microbiota becoming more diverse, start stabilizing [32, 35, 37, 38]. Fecal samples collected from different geographical region

presented that gut microbiota composition taking shape towards adult-like configuration till first 3 years of infants age[32] and post this age gut composition become more persistent [39].

Primarily, Firmicutes and Bacteroidetes phylum dominated the adult human gut microbial composition and Actinobacteria, Proteobacteria and Verrucomicrobia found in lesser preparation. Fecal metagenomic analysis from 4 different countries identified, the well classified robust gut microbial communities named as enterotypes represented through multiple level of 3 genera: *Prevotella*, *Ruminococcus* and *Bacteroides* [40] and this classification of enterotype is independent to the nationality, age, body mass index (BMI), gender. However, this enterotype based classification remain a topic of debate because external factors such as diet, considered as primary regulator of gut microbiota composition and functions[41, 42] and also failed to identified in a healthy and elderly individuals. [43]. Other than diet, aging is also a considerable factor which change the gut microbiota composition. The bacteria belong to the *Bacteridaceae*, *Lachnospiraceae* and *Ruminococcaceae* families are negatively correlated with aging independent to the different geographical region, lifestyle, dietary habits[44-46]. Moreover, healthy aging showed increased microbial richness and higher number of *Bifidobacterium*, *Oscillospira*, *Akkermansia*, and *Christensenellaceae* [45]. Emerging metagenomic empirical evidence advocate that the healthier gut always has more diverse microbiota population and healthy gut is essential to maintain human health[47, 48]."

Comment 2: The commonly used parameters (particularly with the amount of alcohol consumption) for diagnosing the AFLD and NAFLD should be mentioned for the general readers.

Reply: We are thankful to the reviewer for this comment and agree that commonly used diagnostic parameters for AFLD and NAFLD should be mentioned in this article for the general readers. As per the reviewer recommendation, we have included the information about the commonly used diagnostic parameters for AFLD and NAFLD in "**Prognostic and/or diagnostic biomarkers**" section starting from line 511 to 545. It also included their references. Included segment is below.

"Generally, constant alcohol intake more than 60 g per day lead to the alcoholic hepatic steatosis condition which also presented with higher level of liver enzyme such aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and in NAFLD daily alcohol intake is approximately 30g per day. Typically, two to three times higher serum AST level has been observed compared to serum ALT due to alcoholic liver injuries. Patients with AFLD also have higher serum gamma-glutamyltranspeptidase (GGT) level[140]. Similarly, NAFLD also has the non-invasive biomarkers detection protocols such as NAFLD fibrosis score (including age, BMI, AST-to-ALT ratio, , IFG and diabetes, albumin and platelets), FIB-4 index (including Age, ALT, AST, and platelets), FibroTest (including total bilirubin, α2-macroglobulin, γ-glutamyltransferase, haptoglobin, and apolipoprotein A1 corrected for sex and age) etc [141]. These are the common diagnostic parameters used for the AFLD and NAFLD diagnosis."

Comment 3: In the introduction (or in AFLD section) it should also be mentioned briefly how alcohol damages liver.

Reply: Thank you so much for your valuable comments. As per your suggestion, we have added the basic mechanism by which alcohol produced the damage in the liver. This added part is included in the secession "Gut microbiota alteration in AFLD" which is more appropriate place to addon without damaging the flow of the manuscript. This added part starts from line number 209 and ends at line 226. It also included their references. Included segment is below.

"Normally, the liver enzyme alcohol dehydrogenase and ethanol-oxidizing system convert the ethanol to acetaldehyde which is toxic to the hepatic cells. Acetaldehyde is immediately metabolized to the acetate and released to the blood stream and used as a biological fuel by the cells for energy production. In persistent elevated ethanol consumption state, the accumulation of toxic acetaldehyde is increased in the liver which produces the highly reactive molecules that generate an oxidative stress milieu and contribute to the liver injuries [16]. Increased in flow of ethanol in liver, altered the SIRT1 signaling and initiate the fat accumulation in the hepatocytes[57]. Ethanol reduce the SIRT1 expression in liver that leads to the fat accumulation in the liver cells via disrupting the SIRT1 dependent multiple transcriptional factor and co-factor, such as peroxisome proliferator-activated receptor α (PPARα), PPARγ co-activator-1α (PGC-1α), AMP-activated kinase (AMPK), lipin-1, β-catenin, forkhead transcription factor O1 (FoxO1), sterol regulatory element-binding protein 1 (SREBP-1), nuclear factor activated T cells c4 (NFATc4), nuclear transcription factor-κB (NF-κB)[57-59]. Ethanol facilitated the inhibition of SIRT1 which leads to the various signaling network disruption that increases the accumulation of fat in hepatocytes by decreasing the β-oxidation and lipolysis and boosting the lipogenesis and inflammation, and collectively generate the AFLD."

Comment 4: Line 184: " 'anti-inflammatory activity' bacteria" may be replaced with "bacteria carrying anti-inflammatory activity".

Reply: As per reviewers' suggestion "'anti-inflammatory activity' bacteria" replaced with "bacteria carrying anti-inflammatory activity". We are grateful to the reviewer for this valuable suggestion that enhance the understanding of this whole sentence to the readers.

Comment 5: Line 236: "Moreover, fungal infection increased the mortality rate in cirrhosis and alcoholic hepatitis patients". How? Would the author like to propose any possible explanation?

Reply: We really appreciate the reviewer thoughtful comment and inquisitiveness about the fungal infection in liver diseases. Here in this statement, we have presented the summery obtained from few clinical trials. According to these clinical trials outcome, patients having liver diseases with fungal infection has higher mortality rate compared to the non-infective and/or patients having bacterial infection. There could be multiple possibilities for this higher mortality rate such as late or poor diagnosis of fungal infection, poor clinical management of these fungal infection, suppression of healthy gut

microbiota etc. However, substantial underlying mechanism for this higher mortality is still not well known but we think that mycotoxins play a vital role in the increment of the mortality rate but to prove this hypothesis empirical evidence are required.

Comment 6: The author must refer Figure 1 also for AFLD, and not just for NAFLD. It should be cited in the text.

Reply: We apologize for this mistake and very thankful to the reviewer for mentioning this. Yes, Figure 1 is illustrating gut microbes' role in both AFLD and NAFLD therefore we have added the Figure 1 reference in AFLD also in Line number 231.

Comment 7: Throughout the manuscript, the 'Gram' positive and 'Gram' negative bacteria have been mentioned as 'gram' positive and 'gram' negative bacteria. Gram must be in capital.

Reply: We apologize for this error and all the "gram" is changed to the "Gram" in the whole manuscript.

Comment 8: Line 395: "Although gut bacteria control bile acid metabolism, the involvement of intestinal bacteria or other gut microbes in bile acid dysregulation in fatty…" I did not understand what the author actually means by "intestinal bacteria" and by "other gut microbes".

Reply: We are remorseful for including not clearly understandable statement and thankful to the reviewer for indicating this to us. As it is already stated that gut microbiota includes bacteria, archaea, fungi, and viruses (in line numbers 365-366) therefore here in this sentence, "intestinal bacteria" only indicating about the bacterial microorganism and "other gut microbes" includes archaea, fungi, and viruses. We have modified this sentence to reduce the unclarity and included archaea, fungi, and viruses with "other gut microbes" to make this statement more understandable. Here is the new sentence **"Although gut bacteria control bile acid metabolism, the involvement of intestinal bacteria or other gut microbes (including archaea, fungi, and viruses) in bile acid dysregulation in fatty liver patients is not completely understood, and more experimental evidence is required to fill the fundamental gaps**." that starts from line 445 and ends at 448.

Comment 9: Line 424: "Recently, a *Klebsiella pneumonia* strain was identified in a NASH patient fecal sample and was responsible for producing endogenous ethanol and increasing the blood ethanol level without alcohol consumption". Please add reference.

Reply: We are very thankful to the reviewer for highlighting this abatement to the authors. We have added the reference for this statement which can be found in line number 476. However, this statement also has some more results which has been discussed and elaborated after this sentence. Thus, the reference was added in the last at line number 480, previously. Considering the reviewer suggestion which we also think is reasonable and logical thus we have added the reference in line number 476 too.

Comment 10: Throughout the manuscript both "microbiota" and "microbiome" have been used to refer the same thing. I recommend using the term "microbiota" to refer all microbes in a particular niche. The term "microbiome", although often used for the same purpose, it actually means the genetic materials of all microbes.

Reply: We are regretful for inconsistency in using of "microbiota" and "microbiome". As per reviewer suggestion we have change "microbiome" to "microbiota" in whole manuscript as we also think that this change is reasonable and more logical.

Comment 11: I do not find the title appropriate. I request the author to give it a thought. A suggestion: significance of gut microbiota in alcoholic and non-alcoholic fatty liver diseases.

Reply: As per reviewer suggestion, we change the title of the manuscript from "Microbiota Associated Fatty Liver Diseases" to "**Significance of Gut Microbiota in Alcoholic and Non-Alcoholic Fatty Liver Diseases**." We are really gratified to the reviewer for their recommendation on the title.

Comment 12: This is a single author paper and therefore "we" (eg. in the abstract) must be replaced with "I".

Reply: We are very grateful to the reviewer for his/her meticulous and thorough scanning of our manuscript and very delighted to make the changes in manuscript according to his/her recommendations. Like other recommendation, we also want to follow reviewer's this recommendation but as we all know writing a scientific article is not a single person work, it's always a teamwork. Unfortunately, we can assign only one person as a first author as per journal guidelines, but all the authors work as a team and contributed equally to this manuscript. Therefore, it is a very humble request from me as a corresponding author to the reviewer and to the journal editorial group that keep this "we" in this sentence as it is. We really appreciate your consideration.

Reviewer 2:

This article provides an up-to-date information regarding the impact of gut microbiota on fatty liver disease, includes pathophysiologic and clinical component of the mentioned topic, gives prospectives for future investigations and has a great interest for basic scientists and clinicians.

Reply: We convey our best gratitude to the Reviewer 2 for his positive, appreciative, and motivating remarks on our manuscript. We also eco with his opinion that the information included in this manuscript is crucial and very useful for the basic scientists and clinicians. Once again thank you so much for your strong recommendation for publishing this manuscript.

Reply to the Editorial Office's comment

We would like to express our immense gratitude to the editorial office, also to the science editor and the company editor in chief for their valuable time investment in this manuscript reviewing process which helped us to elevate the scientific significance of this manuscript. Moreover, we are agreed with the opinion and the recommendations given by the editorial office. We have taken all these recommendations seriously and made the changes in the manuscript, accordingly. Once again thank you so much for positive feedback and your strong recommendation for publishing this manuscript.