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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 60125

Title: Oral microbiome and pancreatic cancer

Reviewer's code: 05194918
Position: Peer Reviewer
Academic degree: MD
Professional title: Doctor
Reviewer's Country/Territory: Greece
Author's Country/Territory: China

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Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

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SPECIFIC COMMENTS TO AUTHORS

I've enjoyed reading this well written, thorough, important and relevant original article. By focusing on the oral microbiome in patients with pancreatic ductal adenocarcinoma (PDAC), the authors reveal the potential of oral microbiome dysbiosis as a potential non-invasive biomarker for PDAC. The title of the paper precisely states the final conclusions in a comprehensive manner. The abstract is intelligible accurately describing the objectives and the results obtained. The introduction provides a generalized background of the topic that gives the reader an appreciation of the role of oral microbiota in health and disease, along with current evidence regarding the association between oral microbiota and gastrointestinal malignancies. The methods that were used in the study are valid and appropriate for the experiment the researchers carried out. They can also be duplicated because the process of each method was stated in the paper with clarity. The experimental design they used was appropriate to the objective of their study because they were able to have a productive flow of the subsequent methods involved, yielding solid results. Moreover, the several figures and tables greatly aid the visualization of the findings in a more understandable format. The discussion greatly summarizes the results and associates them with appropriate references. In the conclusion the findings are presented to the point. Furthermore, the literature cited is relevant to the study. I have few comments that may further improve the current manuscript before acceptance of publication [Pg = page; Par = paragraph; Line = counts from the 1st line of each corresponding paragraph without its title or subtitle]: Pg #5/Par #1/Line 8: "remains a great challenging" Please change to "remains a great challenge". Pg #5/Par #2/Line 6-8: "Dysbacteriosis...lung cancer" It is mandatory that you briefly explain the general concept of oral or gut microbiota dysbiosis along with mentioning its implication and importance in malignant disease. Pg #5/Par #2/Line 8-10: "Accumulated studies...healthy individuals" How does the abundance of

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microbiota shift in these patients? Furthermore bacterial diversity is a crucial factor of microbiota dysbiosis. How does diversity change in these patients? Pg #6/Par #1/Line 4-6: "Tongue coating...oral microbiota" There are some points to be made here regarding the choice of the sampling method. Among all the oral mucosal surfaces the tongue the is the most populated niche and has a significant impact on other regions in the oral cavity, being a reservoir from which oral bacteria travel around the oral cavity to colonize other regions, facilitated by saliva [1]. Compared with the other parts of the oral cavity, the distinct surface characteristics (fissures, crypts, papillae, saliva) of the tongue coating are prone to the colonization, growth, and proliferation of microbiota [2]. Recent studies have reported that tongue coating microbiome could serve as a stable non-invasive biomarker in gastrointestinal cancer [3], significantly distinguishing patients with pancreatic head cancer from healthy individuals [4]. Furthermore, the microbial communities and intra-personal diversity of the tongue and salivary microbiota have shown high levels of similarity [5]. Hence, according to these data and the fact that swabbing of tongue dorsum is less complicated, more specific, and more cost-effective than saliva collection, why did you choose saliva as the most appropriate sample for the evaluation of oral microbiota? Additionally, it is well-known that cancer patients, especially these with pancreatic neoplasms, present systemic immune dysfunction [6]. Since the human throat is rich with lymphoid tissue (Waldeyer's ring) where immune interactions occur and the oral microbiota greatly interferes with local mucosal or systemic immunity [7], would the swabbing from the oropharyngeal rear serve as a more representative biomarker for distinguishing several features between pancreatic cancer patients and healthy controls? Pg #7/Par #1/Line 5-9: "Participants...enrollment" According to the NIH Human Microbiome Project - Core Microbiome Sampling Protocol A [4] the following exclusion criteria should also be taken into consideration regarding microbiota studies: - Use of any of the following

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drugs within the last 6 months: oral, intravenous, intramuscular, nasal or inhaled corticosteroids; presence of oral disease However, they are not mentioned in the Study Design. Moreover, no specific tool is mentioned regarding the validation (or estimation) of oral health between subjects, which is an important factor when evaluating the oral microbiota. Pg #7/Par #3/Line 3-4: "All the...sterile tube". It should be clarified whether the collection of the saliva was stimulated or unstimulated. Pg #11/Par #2/Line 11-14: "Patients with...unresectable PDAC" It would be nice to add another Table presenting the alterations in diversity between resectable and unresectable PDAC patients. Pg #12/Par #2/Line 5-9: "In addition...without diarrhea" These results regarding the bacterial abundances do not match with the respective ones in Table 3. For example, Prevotella presents greater abundance in patients without jaundice (669.4±384.3) compared to those with jaundice (403.2±310.8) as the mean values suggest, thus they should be revised accordingly. Pg #13/Par #2/Line 1-2: "This prospective...adenocarcinoma" Please clarify how the results reflect and oral dysbiosis. Pg #13/Par #2/Line 9-11: "This provides...collect samples" Could this result also reflect the possible translocation of oral bacteria in the gut microenvironment, as it is already evident in colorectal cancer [8]? Pg #15/Par #3/Line 1: "Our study had limitations" The sampling method of the oral microbiota is also a limitation and it should be mentioned here. Pg #26/Table 4: Please fix the headings of the columns so that the results can be easily compared without confusion. All in all, this study gives a new aspect in the research of PDAC, revealing oral microbiota as a novel biomarker for PDAC detection and possibly prognosis. References 1. Danser MM, Gómez SM, Van der Weijden GA. Tongue coating and tongue brushing: a literature review. Int J Dent Hyg. 2003;1:151-8 2. Seerangaiyan K, Jüch F, Winkel EG. Tongue coating: its characteristics and role in intra-oral halitosis and general health-a review. J Breath Res. 2018;12:034001. 3. Xu S, Xiang C, Wu J, Teng Y, Wu Z, Wang R, Lu B, Zhan Z, Wu H, Zhang J. Tongue Coating

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Bacteria as a Potential Stable Biomarker for Gastric Cancer Independent of Lifestyle. Dig Dis Sci. 2020 Oct 12. doi: 10.1007/s10620-020-06637-0. Epub ahead of print. PMID: 33044677. 4. Lu H, Ren Z, Li A, Li J, Xu S, Zhang H, Jiang J, Yang J, Luo Q, Zhou K, Zheng S, Li L. Tongue coating microbiome data distinguish patients with pancreatic head cancer from healthy controls. J Oral Microbiol. 2019 Jan 28;11(1):1563409. doi: 10.1080/20002297.2018.1563409. PMID: 30728915; PMCID: PMC6352935. 5. Hall, M.W., Singh, N., Ng, K.F. et al. Inter-personal diversity and temporal dynamics of dental, tongue, and salivary microbiota in the healthy oral cavity. npj Biofilms Microbiomes 3, 2 (2017). https://doi.org/10.1038/s41522-016-0011-0 6. Poch B, Lotspeich E, Ramadani M, Gansauge S, Beger HG, Gansauge F. Systemic immune dysfunction in pancreatic cancer patients. Langenbecks Arch Surg. 2007 May;392(3):353-8. doi: 10.1007/s00423-006-0140-7. Epub 2007 Jan 19. PMID: 17235586. 7. Adi Idris, Sumaira Z. Hasnain, Lu Z. Huat, David Koh, Human diseases, immunity and the oral microbiota—Insights gained from metagenomic studies, Oral Science International, Volume 14, Issue 2, 2017, Pages 27-32 8. Koliarakis, I.; Messaritakis, I.; Nikolouzakis, T.K.; Hamilos, G.; Souglakos, J.; Tsiaoussis, J. Oral Bacteria and Intestinal Dysbiosis in Colorectal Cancer. Int. J. Mol. Sci. 2019, 20, 4146.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 60125

Title: Oral microbiome and pancreatic cancer

Reviewer's code: 05048167
Position: Editorial Board
Academic degree: PhD

Professional title: Senior Scientist

Reviewer's Country/Territory: Germany

Author's Country/Territory: China

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Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
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SPECIFIC COMMENTS TO AUTHORS

Wei et al had given a study report concerning the Oral microbiome analysis in pancreatic cancer from a local cohort. They found that a group of microbiomes distributed differently in cancer patients as compared to healthy control. From the sequencing analysis, they declared that saliva microbiome was able to distinguish PDAC and healthy individuals. Interestingly, they mentioned that Leptotrichia may be specific for PDAC for patients living in Sichuan Province, southwest China. Base on their study, they suggested that combined symptom and microbiome evaluation may help in early detection of pancreatic cancer. Overall it is a sound study with impressive microbiome analysis for potential application of early detection of pancreatic cancer. However there are still some pointes need to be further improved. From the innovation of the study, the research team also compared the microbiota Profils difference between the patients with different symptoms and without symptoms, which may help in early detection of pancreatic cancer. Concerning the quality and importance of this manuscript, only saliva sample was collected might be a limitation, and it might be important to compare the microbiome of the cancer patient saliva and the tissue samples. For the data analysis, more information could be further explored from the 16s Seq data. From the methodology, Saliva sample collection was only mentioned that all the subjects were instructed to not eat and drink for 0.5 hour prior to saliva sample collection. Did all patients wash their mouth or brush their teeth before sample collection? Did all sample collection at the same time period in a day? Concerning of the parameters (for instance diet) or the symptoms, it will be better to include standard score or relatively clinical chemistry parameters for a more subjective evaluation. Besides, there are some limitations could be paid more attention: In discussion, it was mentioned that Four known Main periodontal disease contributors: aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia and prevotella intermedia were more

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prevalent in PDAC patients in Fan et al.'s study.thus by collecting patients should they also consider to exclude the patients with periodontal disease? The study did not clarify this issue yet and might be important to check.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 60125

Title: Oral microbiome and pancreatic cancer

Reviewer's code: 01171900

Position: Peer Reviewer

Academic degree: PhD

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Reviewer's Country/Territory: Australia

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SPECIFIC COMMENTS TO AUTHORS

This study attempts to characterise the microbiome in PDAC (resectable and non-resectable) and healthy cohort. Furthermore, this study makes a novel contribution by comparing the microbiome in symptomatic and asymptomatic PDAC population. The method used for sequencing and analysis of microbiome is similar to other studies on this topic. However, I do have a few points that need to be addressed. The major point is how and when the saliva samples are taken especially in healthy controls. The author mention that samples are taken 30 minutes before operation but are these taken in fasting condition all the same time of the day? More importantly are healthy control normalized in term of timing? How variable are the results if samples are taken in different time of the day (i.e. morning vs afternoon or evening)? Are 30 minutes without eating or drinking sufficient? More details are required in the methods section. Other points: 1. In the introduction section, the first paragraph, the authors briefly mention a lack of early detection. I was wondering if it would be a good idea to talk about the existing biomarker (CA19) commonly used in the clinic. 2.The study excludes non-cancers PDAC lesions from sequencing (last line in methods section under research design and participants). The supplementary data comparing the Vellionella specie decline from healthy to resectable to non-resectable PDAC is very well presented. Although the authors do attempt to characterise the changes in the microbiota as PDAC advances, since IPMN can give rise to PDAC, it would be interesting to know how the microbiome changes between IPMN, resectable and non-resectable PDAC vs healthy. 3.

How many patients had resectable and non-resectable PDAC is not clear from the manuscript? 4. I was wondering how many times the symptom data collection was done from PDAC patients in this study. What were the symptomatic differences found in resectable and non resectable PDAC? What was their effect on microbiome from these different cohorts? 5. The symptoms used in the assessment are not mentioned in the

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methods section under subheading phenotype measures. Although the study mentions the list of symptoms under abundance of bacteria and symptom (subheading under statistical analysis), it would be clearer to list to symptoms here as well. 6. From the manuscript, it is not clear how many patients form the symptomatic and asymptomatic cohort. A table on the same can help add more clarity. 7. The manuscript does mention ethnic differences between different Chinese provinces and non-Chinese populations. This study also estimates the lifestyle difference as it mentions 61% of PDAC population has a high-fat diet in comparison to controls. Does this cohort percentage have an increase in specific microbial diversity? 8. Was there any of the microbial differences in the PDAC resectable and non-resectable affected by symptoms? 9. It will be interesting to see whether this microbial diversity is specific for symptomatic PDAC population by looking at previous literature or is it generalizable for the symptom. 10. How to the differences in asymptomatic and symptomatic PDAC population correlate with a healthy cohort? A table summarising the results of the main microbial population will put things in perspective for the reader and make results clearer.