Peer Review File

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Reviewer A

This work proposes a review on the potential salivary biomarkers of several respiratory diseases such as bronchial asthma, COPD, obstructive sleep apnea and so on. This is of course an interesting topic because saliva samples can be collected in a simple and minimally-invasive way. However, the paper has some limitations:

1. It is noted that your manuscript needs careful editing by someone with expertise in technical English editing. Language edition is recommended.

Reply: We regret there were problems with the English. The paper has been carefully revised again to improve the grammar and readability.

2. The major drawback of the manuscript comes from the simplicity of the article. The author only enumerated the potential salivary biomarkers in respiratory diseases. But in our mind, there is no correlation between respiratory diseases and saliva. So, why salivary biomarkers can be used for diagnosis and assessment of respiratory diseases? What is the pathophysiological mechanism of this phenomenon? Is there any research on that?

Reply: Thank you for this valuable feedback. Saliva can be collected in a simple, minimally-invasive, and repeated manner. Recent researches demonstrate that some indicators related to respiratory diseases can be detected in saliva and show statistical differences when compared with health control. Saliva has emerged as a novel diagnostic and evaluation medium for respiratory diseases. However, the pathophysiological mechanism of saliva indicators in respiratory diseases are rarely mentioned. Most of the saliva test results are used for the study rather than the clinical practice. Further studies are needed in the future. We have added some sentences to the Discussion section (page 13, line 251-253), to clarify this.

Changes in the text: Page 13, line 251-253

3. Oral environment is affected by many factors such as diet, smoke history and oral diseases. So, how to ensure the stability of saliva sample collection? Is there any research that provide a standard protocol for saliva collection and saliva preservation? Or is there any research that studied the influence of these factors on the results?

Reply: We are extremely grateful to you for pointing out this problem. Saliva composition is affected by many factors such as diet, smoke history and oral

diseases. There is no standard protocol for saliva collection, which is the limitation of saliva test should be mentioned, not only for respiratory diseases researches, but also for other diseases. Establishing a standard protocol for saliva collection and saliva preservation is a challenge for saliva test in the future. We have added some sentences to clarify this (page 13, line 254-261).
Changes in the text: Page 13, line 254-261

4. How are these markers expressed in blood or other body fluids? Whether or not it is related to the expression in saliva?
Reply: Thank you for this valuable feedback. Recent studies about saliva in respiratory diseases focus on whether the indicators, which mostly have been as detection markers in the serum of respiratory patients, can be detected in saliva, and whether differential expression levels of saliva indicator can be used as potential biomarkers of disease. Some studies mention the relationship of saliva and serum expression of some indicators. However, for these biomarkers, differences between saliva values and known serum values need further study to examine whether saliva samples can be a clinically diagnostic tool. We have added some sentences to clarify this (page 13, line 261-267).
Changes in the text: Page 13, line 261-267

5. For Figure 1, what is the basis of classifies as promising, inconclusive and negative?
Reply: Thank you for pointing out this problem. For each one of respiratory diseases, a categorical system classifies the biomarkers as: promising, inconclusive and negative results based upon the findings of our work. All variations reported are in comparison to healthy controls, if biomarker variation is specific to disease or illness severity, then it is stated as promising. If the biomarker shows no difference between patients and health controls, it is stated as negative. If the result is inconclusive or ambiguous, it is classified as inconclusive.

6. In addition, the list of references is not uniform. For example, most of the journal names in references are showed in abbreviation but a few are showed in full name, such reference 13, 22 and 26. Please recheck the format of the references.
Reply: Thank you for this valuable feedback, we have modified reference as advised (see Page 24-28, line 316-509).
Changes in the text: Page 24-28, line 316-509

Reviewer B

In this review, the authors have summarized current studies on salivary biomarkers in respiratory diseases. Though the review is interesting, the authors need to mention some of the contents in detail.

1. For instance, saliva CRP has a high specificity in pneumonia, so what is the difference between different types of pneumonia with saliva CRP?

Reply: Thank you for pointing out this problem. Salivary CRP could be an alternative biomarker for serum CRP in pneumonia diagnosis, especially for pediatric patients. However, the value of saliva CRP for different types of pneumonia is rarely mentioned. We have added some sentences to clarify this (page 9, line 165-176).

Changes in the text: Page 9, line 165-176

2. And which pathogens in saliva before operation can predict the risk of postoperative aspiration pneumonia? The specific bacterial richness and diversity in saliva periodontitis patients with COPD need to be added for further review.

Reply: Thank you for pointing out this problem. Salivary microbiomes are rarely studied in COPD and pneumonia. The references in our paper focus on the relationship of periodontitis and COPD or pneumonia, specific oral bacterial associated with COPD or pneumonia was not provided.

3. The microbiome of bronchial asthma and lung cancer is different from that of normal people, what are the changes in the development of the disease and what is the mechanism between these microorganisms and the disease? Discussion on these aspects are necessary.

Reply: Thank you for this valuable feedback. The pathophysiological mechanisms between these changes of salivary microorganisms and the development of asthma or lung cancer were rarely mentioned, further studies are needed. We have added some sentences to clarify this(Page 6, line96-98; Page 12, line 227-231).

Changes in the text: Page 6, line96-98; Page 12, line 227-231

Reviewer C

The manuscript JTD-21-202-CL-RV17-1211 is a very complete bibliographic revision of the current state of the saliva as a source for the search of biomarkers associated to respiratory diseases. This field is important since saliva is one of the less invasive and more easy to collect biological fluids and the development of techniques to detect infectious diseases as MDR-TB or SARS-CoV-2 using saliva as source is currently of paramount importance.

I only have some minor concerns:

1. Line 11: I assume the authors mean "DNA" instead of "gene". Please change.
Reply: Thank you for this valuable feedback. We have modified the expression as advised (Page 3, line 36).
Changes in the text: Page 3, line 36.

2. Line 21: I am quite sure saliva contains much more than 400 proteins. The numbers of course always depend on the sensitivity of the technique used. For instance, recently, more than 800 different proteins were identified in saliva using quantitative shotgun proteomics (Mateos et al., JOP 2019). Please update references and use a more scientific term as "are detected in" rather than "containing".
Reply: Thank you for this valuable feedback. We have modified the expression as advised (Page 4, line 46-50).
Changes in the text: Page 4, line 46-50.

3. I miss some previous references as a very comprehensive review published some years ago (Ruhl et al., Expert Rev Proteomics 2014).
Reply: Thank you for this valuable feedback. We are sorry that we did not find the reference, just find (Ruhl et al., Expert Rev Proteomics 2012). We have added the reference as advised (Page 4, line 51,58,reference10).
Changes in the text: Page 4, line 51,58,reference10.

4. Specifically in TB, in the previous mentioned study (Mateos et al., JOP 2019) the authors detected a differential increased proteomic signature of active TB patients versus infected (LTI) and uninfected contacts (acute phase-related proteins like haptoglobin and fibrinogen) but also a decrease in active TB patients of proteins related to carbohydrate metabolism, which was also suggested previously by NMR (Shin et al., JPR 2011 and Zhou et al., JPR 2013), Please update and discuss accordingly.
Reply: Thank you for this valuable feedback. We have added the reference (Mateos et al., JOP 2019) as advised (Page 10-11, line 193-199). Although we agree with you that method NMR offers various advantages in saliva studies, we don’t add the references (Shin et al., JPR 2011 and Zhou et al., JPR 2013) since saliva test was not involved in Zhou’s study, and Shin’s research was on rats. If you require further discussion of these studies, we will be happy to add a supporting paragraph to the paper.
Changes in the text: Page 10-11, line 193-199.

5. Table 1 should include the technique used for the putative biomarker detection (ELISA, WB, Quantitative Shotgun Proteomics, Targeted Proteomics, Antibody-based Multiplexing, etc).

Reply: We are extremely grateful to you for pointing out this problem. Although we agree with you that technique should be involved in Table 1. Since we focus on the biomarkers but not detection method in our paper, techniques are not added in Table 1. If you require further notes of techniques involved in these researches, we will be happy to add.

Changes in the text: No.

Reviewer C-Re-review

The authors have satisfactorily answered some of my concerns in this version of the manuscript (JTD-21-202-R1). However they missed to address the most important suggestion which is to complete Table 1 with the technique used for each biomarker discovery study. The biological significance of the biomarkers are defined in part by the technique used, is not the same to propose a new biomarker using WB than using for instance shotgun quantitative proteomics followed by verification by PRM or ELISA.

Reply: We are extremely grateful to you for pointing out this problem. We added some data about technique in table 1.