Author's response to reviews

Title: Soluble toll like receptor 2 (TLR-2) is increased in saliva of children with dental caries.

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Author's response to reviews: see over
Dear Professor Braga:

We are delighted that the reviewers have recommended our manuscript for publication in the open source “BMC Oral Health”. Enclosed you will find the final revised manuscript entitled “Soluble toll like receptor 2 (TLR-2) is increased in saliva of children with dental caries” by Alyssa Zhao, Corinne Blackburn, Judith Chin and Mythily Srinivasan.

We appreciate the time and effort spent by the reviewers and the editor in going over our manuscript. The changes to the paper are noted below preceded by the reviewer’s comments in italics.

Referee report and our response:

**REVIEWER 1 EVALUATION**

**Final comments:**

The presented study demonstrates higher contents of sTLR-2 in caries active saliva compared to children without caries. Authors suggested that salivary sTLR-2 level may represent an efficient biomarker for caries activity. I think that correlation between sTLR-2 levels with cariogenic bacterial counts will be necessary for further analysis. It is important subject for researchers, because findings from experiments will help in prevention and treatment of caries. This article falls within the scope of the journal. It is well conducted and explores important issues. This is an acceptable article, but after minor essentials revisions.

**Response:** We are thankful for the encouraging comments by the reviewer. We agree with the reviewer that correlation between salivary sTLR-2 levels with cariogenic bacterial burden is needed for definitive characterization of sTLR-2 as a marker for caries risk.

The following sentences in the manuscript acknowledge the need for further studies to address this critical concern.

In the “Results and Discussion” section (Pg 8- lines 10-18)

“Based on its strong association with caries incidence multiple studies evaluated salivary levels of S.mutans for caries risk prediction with variable results [1, 29]. The polymicrobial etiology of dental caries as well as the interaction between salivary proteins and S.mutans could contribute to the variability [26, 30]. Four major types of salivary protein-microbe interaction have been observed in vitro. These include aggregation, adherence, inhibition/cell-killing, and nutrition [30]. It is postulated that the observed increase in sTLR-2 in caries active saliva may represent a host measure to combat the increased Gram +ve cariogenic bacteria. This suggests that the salivary sTLR-2 level may represent an efficient biomarker for caries activity. The wide variation in the sTLR-2 concentration in caries active saliva could be due to the extent of caries.”

Page 9 lines 1-2

“Future studies will correlate the sTLR-2 levels with the cariogenic bacterial counts in saliva”.

**Specific comments (Minor essential revisions):**

*Critique 1:* The title is correct and in accord with abstract, but IL-8 should appear also in abstract, because it was described in Methods, the level of IL-8 was presented in Fig.1B and in section: Results and Discussion.
The concentration of IL-8 was showed in Fig.1B, but detailed characteristic of that proinflammatory cytokine was missed in Background section. It should be completed.

Response: We appreciate the reviewer’s concern. The abstract is modified as follows:

Methods: Here we investigated the association between dental caries and two soluble proteins known to function at the host microbe interface, sCD14 and sTLR-2, as well as the cytokine IL-8 in saliva by enzyme linked immunosorbent assay.

Results: While the level of sCD14 and that of IL-8 was equivocal between the two groups, the sTLR-2 concentration in caries active saliva was significantly higher than that in caries free saliva.

The following are included in the “Results and Discussion” section. Page 7: lines 21-22, Page8: lines 1-3.

“Previously Gornowicz et al., have reported elevated levels of salivary IL-8 in dental caries [28]. We observed that the salivary IL-8 concentration did not differ significantly between caries free and caries active saliva (Fig 2A). The variable observation between the two studies could be attributed to the differences in the age and the nature of the sample (amylase and Ig depleted vs. unpdepleted)”.

Critique 2. In section Methods the substrate which was used for HRP is TMB (3,3',5,5'-Tetramethylbenzidine), not TNB.
Response: We are thankful for the reviewer’s critical observation. The mispelled abbreviation is corrected.

Critique 3. In section Methods the authors said: „The concentration of sTLR-2 or sCD14 in pg/mg of salivary proteins was determined using standard curve of purified recombinant human CD14Fc and TLR-2Fc of known concentration”. The sentence should be corrected, the results were presented in pg/mL (Fig.2A), but in section Results authors used „ng/ml”. The clarifications are needed.
Response: We appreciate the reviewer’s astute observation. The editorial errors are corrected. The relevant sentences in the revised manuscript are given below.


“Bound CD14 and TLR-2 was detected with horse radish peroxidase (HRP) conjugated anti-mouse IgG followed by TMB substrate (Pharmingen, San Diego, CA). Absorbance at 450nm was read in a miscorrelate reader (model 680: Biorad Laboratories, CA). The concentration of sTLR-2 or sCD14 in pg/ml of salivary proteins was determined using a standard curve of purified recombinant human CD14Fc and TLR-2Fc (R&D Systems) of known concentration.”

Page 8: lines 3-5.

“The concentration of sCD14 in saliva was equivocal in both groups; ranging between 509pg/ml and 1443pg/ml in caries free group and between 609pg/ml and 1829pg/ml in caries active group (Fig 2A)”.

REVIEWER 2 EVALUATION

Final comments:
The paper presents the possibility of using a new salivary marker for dental caries. The paper is relevant and this article is well written and grounded.
Response: We appreciate the reviewer’s comments.

Minor Essential Revisions:
Critique 1: The authors could discuss a possible correlation between number of caries lesions and the amount of the salivary marker. If this salivary marker could also predict the severity of the disease.
Response: We agree with the reviewer’s comment. Please see our response above to the final comments of Reviewer-1.