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 Morawska:

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 concerns of the editor in going over our manuscript. The changes to the paper are noted below preceded by the reviewer's comments in italics.

Editor's comments and our response:

Editorial comments:

1) Thank you for this submission and your revisions. However, we feel that the rationale for this study is not well explained and it would much improve the paper if you could expand the grounds for conducting this study in the background section.

Response:
We changed the “Background” section in the revised version significantly. Changes made to the manuscript are highlighted in the “Revised and Marked” file. It is also copied below. The blue and yellow highlighted scripts represented contents that were re-arranged and newly added respectively.

Saliva is recognized as a rich source of host factors capable of modulating the caries process [1, 2]. Technological advancements have aided in the characterization of salivary proteomics and peptidomics with the identification of 1444 proteins and 11893 peptides respectively [3]. These protein/peptides belong to different functional classes such as those involved in response to stimulus/stress, antioxidant functions, catalytic functions and enzyme regulators [3, 4]. Several salivary components have been assessed for an association with dental caries. While some exhibit weak association, others were equivocal between normal and caries active saliva [4-7]. Assessment of salivary glycoproteins with specific oligosaccharides showed that higher levels of select oligosaccharide that facilitate bacterial colonization at the surface of teeth correlate with caries incidence in young adults [8]. Salivary levels of antimicrobial agents such as alpha defensins, statherin and cystatin S have been suggested as potential risk factors for caries development[5, 9].

Toll like receptors (TLR) are germ line encoded receptors that recognize conserved microbial patterns typically shared by large groups of microorganisms. Currently 13 mammalian TLRs and many of their ligands are known [10]. Functioning either alone or in concert with specific co-receptors in recognizing microbial patterns the TLRs act as gate-keepers constantly sampling the environment and eliciting responses to prevent/control infection [10, 11]. TLR-2 and TLR-4 have been shown to recognize the peptidoglycan of Gram positive and the lipopolysaccharide of the Gram negative bacteria respectively either alone or in association with the common co-
receptor CD14\([12, 13]\). Odontoblasts localized at the dentino-pulpal surface in healthy teeth have been shown to express TLR-2 and TLR-4. Depending upon the nature of the odontoblastic response caries progression is either suppressed with the formation of reactionary dentin or accelerated leading to pulpal inflammation\([14, 15]\). Microbial invasion of dentin has been shown to upregulate TLR-4 in odontoblasts and mediate TGF-\(\beta\) secretion facilitating collagen synthesis. In addition TLR-4 signaling in odontoblasts also upregulate matrix metalloproteinase-2 promoting cleavage of dentin sialophosphoprotein (DSPP) to dental sialoprotein (DSP) which forms a nucleation site for hydroxyapatite crystal formation in the newly formed collagen\([14]\). Stimulation of odontoblast like cells with cell wall components of Gram-positive bacteria elicited cytokine and chemokine secretions in a TLR-2 dependent manner\([15]\). Elevated levels of cytokines IL-6, TNF-\(\alpha\) and IL-8 have been observed in caries active saliva\([16]\). While primarily membrane associated, recently soluble forms of certain TLRs have been identified in body fluids. It has been suggested that the soluble TLRs function to sequester pathogens\([17-20]\). Recently, we and others have reported the presence of soluble sCD14 and sTLR-2 in saliva\([19, 21]\). TLR-2 has been shown to recognize the peptidoglycan and the lipoteichoic acid of \textit{Streptococcus mutans}, the most common cariogenic bacteria\([22]\). Considerable evidence suggests a strong correlation between the increased presence of cariogenic bacteria in the plaque biofilm and elevated numbers of the same bacteria in saliva\([1, 5]\). Hence we hypothesized that the level of sTLR-2 and sCD14 in the saliva of caries active individuals will yield an indirect measure of the bacterial burden and act as a biomarker of caries activity. Our data suggest that the sTLR-2 is higher in the unstimulated whole saliva (UWS) of children with active caries lesions.

Response: The abstract has been considerably modified as follows. The changes are highlighted in yellow.

**Background:** Dental caries is the most common microbial disease affecting mankind. Caries risk assessment methods, identification of biomarkers and vaccine development strategies are being emphasized to control the incidence of the largely preventable disease. Pattern recognition receptors such as the toll like receptors (TLR) have been implicated as modulators of host-microbial interactions. Soluble TLR-2 and its co-receptor, CD14 identified in saliva can bind the cell wall components of cariogenic bacteria and modulate the disease process. The objective of this study is to determine the potential of salivary sTLR-2 and sCD14 as biomarkers of caries activity and indirect measures of the cariogenic bacterial burden.

**Methods:** Unstimulated whole saliva was collected from twenty caries free and twenty caries active children between the ages of 5 and 13 years. The concentration of sCD14 and sTLR-2 together with that of the cytokine IL-8 reported to be increased in dental caries was assessed by the 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.

Conclusions: The sTLR-2 in saliva could serve as a potential biomarker for caries activity.

3) Please include all authors emails on the title page.

Response: The e-mails of all authors are included in the title page of the revised text.

4) Please clarify if your study was approved by the institutional review board, the wording at the moment does not clearly state that.

Response: The following change is made in the “Methods” section.

The study was approved by the institutional review board of the Indiana University Purdue University at Indianapolis.

We thank the editor for the careful review and critiques. We hope that the manuscript is now acceptable for publication.

Sincerely
Mythily Srinivasan