Author's response to reviews

Title: Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in patients with intellectual disabilities

Authors:

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Author's response to reviews: see over
Reviewer's report

Title: Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in patients with intellectual disabilities

Version: 2 Date: 13 May 2015

Reviewer: Georg Conrads

Reviewer's report:

In their manuscript entitled “Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and *S. sobrinus* in patients with intellectual disabilities” Oda et al. investigate the prevalence and incidence of two cariogenic species, *Streptococcus mutans* and *S. sobrinus* in 145 mentally disabled young individuals (age 6-30) applying end-point PCR.

Major Compulsory points

The study population is quite interesting but their mental handicaps are not specified or “quantified”. They were followed longitudinally over one year in their caries and caries-agent quantities but the method applied (end-point PCR) is a kind of obsolete.

The English language needs improvement.

Response to reviewer: The subjects were defined, and I added a Table. I also described the some sentences in Methods section.

Minor Essential points

Background line 4: the term “pathogen” for mutans-streptococci is obsolete or –at least- needs some modification or reduction. I recommend to use “putative pathogen (or parasitic symbiont according to the extended ecological plaque hypothesis [please cite Takahashi & Nyvad 2011])”.

Response to reviewer: According to the reviewer’s suggestions, I changed the term of putative pathogen instead of pathogen. I cited the review by Takahashi & Nyvad in the text.

Line 10-11: please correct and cut, I recommend “as they have been shown to be capable of detecting low numbers (5-100) of bacterial cells”

Response to reviewer: I changed the sentence according to the reviewer’s suggestion.
Line 24: should read “these two species and caries activity in children…”

Response to reviewer: I changed the sentence as “these two species and caries activity in children….”

Methods, line 2: Please provide how your ID subjects were chosen/scored as being intellectual disabled. How many had down-syndrome etc. Provide a table with a few clinical entities and numbers of patients. Very important!

Response to reviewer: I added a Table of study subjects and described the subjects with ID defined and the rate of ID and Down syndrome in the Methods section.

Line 2: “old” is redundant.
Response to reviewer: According to the reviewer’s suggestion I deleted “old”.

Line 13-14: Please give further details of the method for sampling; did they brush with a “dry” toothbrush or was any liquid used; the reference cited is subjecting periodontopathogens (subgingival bacteria). Hard to believe that the protocols can be transferred 1:1 for the collection of supragingival samples.

Response to reviewer: I re-described “Dental plaque was collected from all erupted teeth by brushing with a sterile toothbrush for 1 minute using a previously described method [18]. During toothbrushing, plaque adhering to the toothbrush was removed by washing several times in a tube of sterile distilled water. The plaque samples in the tube were immediately transported to our research laboratory and stored at –20°C, prior to extraction of genomic DNA.”

I would not mean the sub-gingival or supra-gingival plaque to collect plaque samples by a toothbrush. It was difficult to collect saliva samples from individuals with ID, it was rather easy to take plaque samples from the subjects by toothbrushing as much as possible.

Lines 20ff: please use forward and reversed instead of upper and lower and “complementary” instead of “complimentary” (very different meaning). Please provide a sentence about the principal target of these species specific PCRs (dextranase gene).

Response to reviewer: I change the sentences in the Methods section according to the reviewer’s suggestion as follows: *Streptococcus mutans* JCM5175 and *S. sobrinus*
ATCC27607 were used as control species. PCR detection of the target species was performed using primers described by Igarashi et al. [7,9]. Oligonucleotide primers were designed to the *dex* DNA sequence of *S. mutans* (GenBank accession no. D49430) and *S. sobrinus* (GenBank accession no. M96978). For *S. mutans*, the forward primer, 5’ TAT GCT GCT ATT GGA GGT TC 3’, is complementary to the sequence 973-992, and the reversed primer, 5’ AAG GTT GAG CAA TTG AAT CG 3’, is complimentary to the sequence 2225-2244. For *S. sobrinus*, the forward primer, 5’ TGC TAT CTT TCC CTA GCA TG 3’, is complementary to the sequence 134-153, and the reversed primer, 5’ GGT ATT CGG TTT GAC TGC 3’, is complimentary to the sequence 1726-1743.

Page 6, line 2ff: please give the ratio for the A. actinomycetemcomitans-directed PCR; this was done for proofing absence of PCR inhibiting substances (and principal presence of bacteria, proof that the brushes were used. It is a bacteria-universal PCR with A.a. as a reference target only. This must be explained to the reader. Again, complementary and forward, reversed must be used.

**Response to reviewer:** I changed the sentences; “The primers for eubacteria 16S ribosomal RNA sequence (GenBank accession number M75035) were used to confirm the presence of bacteria in plaque samples as positive control [19]. The forward primer, 5’ CAG GAT TAG ATA CCC TGG TAG TCC ACG C 3’, is complementary to the sequence 783-810, and the reversed primer, 5’ GAC GGG CGG TGT GTA CAA GGC CCG GGA ACG 3’, is complementary to the sequence 1378-1407. The size of the expected PCR product was 625bp”.

Results, line 7: “at after 1 year“ redundant preposition

**Response to reviewer:** I deleted “at”.

Lines 12-13 (and later page 10, line 19 as well as page 11 line 14 and end of discussion, Table 2): “increases in caries increment“ seems to be redundant; is this phrase really in use or do you mean increase in caries prevalence which is “incidence”. You are using the correct terms in your title. Please reconsider phrasing.

**Response to reviewer:** I changed “caries incremental increases” instead of “increases in caries increment” in the results, discussion section and table 3 (new Table number).
Discussion, line 13ff: “To detect S. mutans and S. sobrinus in the present subjects, we performed PCR assays with eubacterial 16S rRNA-based primers using dental plaque samples obtained by a toothbrushing method, which confirmed the presence of bacterial DNA in all plaque samples (data not shown).” This sentence is unclear. I recommend “To ensure presence of a representative bacterial sample in all cases and absence of PCR inhibiting substances we performed a broad-range PCR assays applying eubacterial 16S rRNA-based primers and subjecting all samples obtained by the toothbrushing method. This confirmed the presence of bacteria and bacterial DNA in all plaque samples (data not shown).”

**Response to reviewer:** I changed the sentences according to the reviewer’s suggestion.

Line 18ff: the sensitivity testing method has to move to the M&M section. Lines 23-line 2 next page: results are repeated which should be avoided.

**Response to reviewer:** I moved the sensitivity sentence to the Methods section. I also deleted the results sentence that the reviewer indicated.

Final comments:

After a major revision this article could be appropriate for publishing in BMC Oral Health; however the authors should establish real-time quantitative PCR for future studies. This reviewer wonders why in all studies of this particular group (and in contrast to almost all other studies worldwide) the prevalence of S. sobrinus (with or without S. mutans) is so high. For studying the synergistic potential of both species this reviewer recommends to read a recent review published in Journal of Oral Microbiology comparing both species on whole genome level.

**Response to reviewer:** We are planning to investigate the caries risk associated with *S. mutans* and *S. sobrinus* using real-time quantitative PCR for individuals both strains possessed. We agree with your suggestion, and we wonder why prevalence of *S. sobrinus* was high in the individuals with ID. I think this assay is correct to detect the presence of target bacteria. Finally, your review published in the Journal of Oral Microbiology was very helpful for the future research.

**Level of interest:** An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: no competing interests
Reviewer's report

Title: Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in patients with intellectual disabilities

Version: 2

Date: 9 June 2015

Reviewer: John Butcher

Reviewer's report:

BMC Oral Health Review

Major compulsory Revisions

1. The study incorporated 145 patients with ID between the ages of 6 and 30. ID is a very broad term. The condition needs to be defined.

2. This age range is large, can these individuals be classed in the same group, see comment 1.

Response to reviewer: The subjects were defined, and I added a Table. I also described the some sentences in Methods section.

3. The Igarashi paper cited does not describe a primer set for detection of S. mutans – there is therefore no evidence to the validate this primer set. Include an appropriate citation or show the PCR as a figure / supplementary figure.

Response to reviewer: I change the sentences in the Methods section according to the reviewer’s suggestion as follows: *Streptococcus mutans* JCM5175 and *S. sobrinus* ATCC27607 were used as control species. PCR detection of the target species was performed using primers described by Igarashi et al. [7,9]. Oligonucleotide primers were designed to the *dex* DNA sequence of *S. mutans* (GenBank accession no. D49430) and *S. sobrinus* (GenBank accession no. M96978). For *S. mutans*, the forward primer, 5’ TAT GCT GCT ATT GGA GGT TC 3’, is complementary to the sequence 973-992, and the reversed primer, 5’ AAG GTT GAG CAA TTG AAT CG 3’, is complimentary to the sequence 2225-2244. For *S. sobrinus*, the forward primer, 5’ TGC TAT CTT TCC CTA GCA TG 3’, is complementary to the sequence 134-153, and the reversed primer, 5’ GGT ATT CGG TTT GAC TGC 3’, is complimentary to the sequence 1726-1743.
4. There is no good reason for including the 16S rRNA information – none of the data was shown and the method is not novel. The reference for this primer set is also wrong, or did the authors design this primer set? Clarify.

**Response to reviewer:** I changed the sentences; “The primers for eubacteria 16S ribosomal RNA sequence (GenBank accession number M75035) were used to confirm the presence of bacteria in plaque samples as positive control [19]. The forward primer, 5’ CAG GAT TAG ATA CCC TGG TAG TCC ACG C 3’, is complementary to the sequence 783-810, and the reversed primer, 5’ GAC GGG CGG TGT GTA CAA GGC CCG GGA ACG 3’, is complementary to the sequence 1378-1407. The size of the expected PCR product was 625bp”.

5. Both tables are poorly formatted. Amend.

**Response to reviewer:** I amended Table 2 and Table 3 (new number). I hope you are satisfied.

The authors state in the discussion that quantitative studies are required. I am in agreement with this, but I am of the opinion that quantitative studies need to be included to make this manuscript suitable for publication. Without them there is little value to the present manuscript. As the authors are aware, PCR detection of S. mutans and S. sobrinus in dental plaque has been routine for over a decade. The only novel factor to the manuscript appears to be the population tested, which in itself is poorly defined and over a large age range.

The research does not constitute a useful contribution to the field.

**Response to reviewer:** The subjects were defined, and I added a Table of study subjects and described the subjects with ID defined and the rate of ID and Down syndrome in the Methods section.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** I declare that I have no competing interests
Reviewer's report

Title: Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in patients with intellectual disabilities

Version: 2 Date: 10 June 2015

Reviewer: Cristiane Y Koga-Ito

Reviewer's report:
The present study aimed to detect S. mutans and S. sobrinus among Japanese patients with ID using a PCR method, and then compared their presence with the incidence of dental caries over a 1-year period. The subject of the study is interesting, however some methodological doubts exist.

Major compulsory revisions
Authors reported that “Several investigators have also developed polymerase chain reaction (PCR) methods and reported them to be more sensitive for detection as compared to conventional culture techniques”. Specific PCR assays are able to detect the presence of the bacteria but do not give information about the bacterial load or cell viability. Previous published studies indicate that high caries risk is positively correlated to high counts of cariogenic bacteria. Do the authors believe that the absence of bacterial counts can be considered a limitation of the study? Please, discuss.

Response to reviewer: Our conclusion is that individuals with ID harboring both S. mutans and S. sobrinus have a significantly higher incidence of dental caries than those positive for S. mutans alone. We did not investigate the amount of bacterial counts in plaque sample collected by toothbrush. I just wanted to note the co-infection of S. mutans and S. sobrinus has a higher incidence of dental caries than the single infection in general population and intellectual disability in both children and adults. I understand that many reports show that individuals with intellectual disability have poorer oral hygiene and have more dental disease than general population. The oral health status of individuals with ID is related not only to their cognitive patterns and developmental anomalies, but also related to many factors, including age, type of caregiver, and physical disability. Because of those many factors, it could not be easy to improve the oral environment of a patient with ID. However, it is necessary to develop prevention and treatment protocols to improve oral health outcomes of adults with ID. So, present results are a clue to promote prevention of dental caries for individuals with ID in the
future. I agree with your suggestion. We are planning to investigate the caries risk associated with *S. mutans* and *S. sobrinus* using real-time quantitative PCR for individuals both strains possessed. However, initially screening caries risk for patients with ID, it is easy to apply the conventional PCR from the plaque. Detail investigations for dental caries risk are needed in the future using real-time quantitative PCR, and reveal the mechanism of caries risk in co-infection. We discussed those in the Discussion section.

The authors observed that “Four (21.1%) of the subjects with *S. mutans* alone, 9 (34.6%) with *S. sobrinus* alone, and 54 (55.7%) with both had increases in caries increment, while none of the subjects possessing neither organism showed an increase in increment.”. Caries is a very complex and multifactorial disease. At what extent, do the authors believe that other predisposing factors, besides streptococci presence, interfered in the caries incidence outcome? For instance, what was the influence of diet and biofilm control in this outcome? Please, discuss and consider including information about the other predisposing factors (i.e. dietary data, severity of the disability and difficulties in dental brushing, sugar-containing medication intake, salivary flow, oral breathing, use of orthodontic devices). The other variables seem to be very important and should be considered, in particular, considering that this is a longitudinal study that evaluated the patient after 1 year from baseline.

**Response to reviewer:** I agree with your opinion. I think that the initiation and progression of dental caries occurs due to the interaction of three factors: oral microorganisms, dietary carbohydrates and tooth enamel. However, we focused on the evidence that bacterial co-infection mutans streptococci strongly influenced the caries incremental increase in individuals with ID. If we have a chance of multiple factors survey of dental caries risk, we would do it.

In the discussion section, the authors reinforced the importance of choosing a suitable technique for caries prevention based on the risk among patients with ID. At what extent the findings of this study can be applied in the clinical practice? Do the authors propose a microbiological marker to high caries risk (the presence of both *S. mutans* and *S. sobrinus*)?

**Response to reviewer:** It is important to know the bacterial transmission to children in
early stage for prevention of dental caries. However, since we have reported in the papers that children already possessed with both \textit{S. mutans} and \textit{S. sobrinus} could have a high caries risk, we will try to develop new caries prevention strategy, not only to improve oral hygiene and re-mineralization of teeth by fluoride but also to improve the oral flora of low caries activity using the replacement therapy.

Considering that the age range was very wide (6 to 30 yrs-old), DFT can be deeply influenced by the - past experience of caries- factor, in particular in older patients. We would like to suggest the comparison of groups (with \textit{S. mutans}, with \textit{S. sobrinus}, with both or without both) inside more homogeneous age groups (for instance, children, young adults, adults, as proposed by WHO).

**Response to reviewer:** Indeed, I agree with your comment which the age range was very wide. I added a new Table of study subjects and described the subjects with ID defined and the rate of ID and Down syndrome in the Methods section by other reviewer’s suggestion. However, we can conclude that individuals with ID harboring both \textit{S. mutans} and \textit{S. sobrinus} have a significantly higher incidence of dental caries than those positive for \textit{S. mutans} alone through children to adults.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** I declare that I have no competing interests’ below.