Author’s response to reviews

Title: Prevalence of Epstein-Barr virus DNA and Porphyromonas gingivalis in Japanese peri-implantitis patients

Authors:

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Author’s response to reviews:

Dear Sir,

I am sending herewith a revised version of the manuscript entitled “Prevalence of Epstein-Barr virus DNA and Porphyromonas gingivalis in Japanese peri-implantitis patients” by Ayako Kato, Kenichi Imai, Hiroki Sato and Yorimasa Ogata.

The manuscript consists of 23 pages, 6 tables (OHEA-D-17-00300.R1).

We have considered each point raised by the reviewers and have revised the manuscript accordingly. A list of the changes, addressing the reviewer’s points, can be found below. (We highlighted the changes within the document by using red colored text). Of note, some of the points made would require additional experiments, which are currently being addressed in ongoing studies. Since their inclusion would increase the length of this manuscript beyond a reasonable length, we believe it is better that we complete this work as part of a follow-up study.

We believe that the changes that we have made in response to the constructive comments of the reviewers, have improved the presentation of this study, which we hope will now be considered acceptable for publication in BMC Oral Health.
Very sincerely yours,

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Reviewer 1 (Dr. Yuichiro Kikuchi)

(Response)

Thank you very much for your valuable comments.

Major Compulsory Revisions

1. Pay attention to proper use of the English language. There are many grammatical errors or typographical errors and the sentences could be re-constructed to be of better clarity. English in this manuscript may need editing service.

(Response)

According to the reviewer’s suggestion, we revised English language. We truly appreciate the reviewer for giving us this comment.

2. Many abbreviations, such as "PI", "PPD", "BOP" and "SUP" are written in the manuscript. It is difficult to understand the text or some Tables mean at a glance. List of abbreviations should be included in the main text.

(Response)

According to the reviewer’s suggestion, we revised text and tables, and added the list of abbreviations in the main text (page 15).
3. (Methods)

Subjects and subject distribution

I think it is not possible to simply compare HI and PI if the periods of implants loading time of HI and PI are very different. Please explain the duration of periods of implants loading time of HI and PI.

(Response)

According to the reviewer’s suggestion, we added periods of implants loading time of HI and PI in Table 3 and 4. Loading time of healthy implant group and PI group were 4.45 ± 2.53 years (mean ± SD) and 5.63 ± 2.72 years, respectively (Table 3 and 4). Mean loading time of PI group was longer than healthy implant group. However, there was no significant difference between two groups. Therefore we added description of loading time in the results (page 9, lines 3-6).

4. (Methods)

Page 7 Ins 22-23

I do not understand the sentence "the expressions of EBV DNA and P. gingivalis relative to GAPDH were determined". Why do the authors determine the expression of P. gingivalis using the GAPDH? Please clarify.

(Response)

Thank you very much for your valuable comment. We used GAPDH only for the expression of EBV. Therefore, we revised the description of Page 7 Ins 22-23 from “Post-PCR melting curves confirmed the specificity of single-target amplification, and the expressions of EBV DNA and P. gingivalis relative to GAPDH were determined.” to “Post-PCR melting curves confirmed the specificity of single-target amplification, and the expressions of EBV DNA and P. gingivalis relative to GAPDH were determined.”

Minor Essential Revisions

1. (Keywords)

Change "P. gingivalis" to "Porphyromonas gingivalis".

(Response)
We have changed (Page 2).

2. (Background)
Page 3 Ins 32-33
I do not understand the meaning of the word "microbial interactions". EBV is a virus. Is "microbial" need? Rewrite sentence for more clarity. Similar errors appear in the manuscript on Page 12 lines 22-23.

(Response)
We have deleted "microbial" on Page 3 and 12.

3. (Methods)
Page 6 Ins 29-36
Change "Kato et al., 2015" to the reference number 17. Similar errors appear in the manuscript on Page 6 lines 32-36.

(Response)
We have changed (Kato et al., 2015) to [17], and (Takada et al., 1991; Watanabe et al., 2011) to [34, 35].

4. Page 7 Ins 9-10
I do not understand the meaning of the words "0.2 μl and 0.4 μM forward and reverse primers". Please clarify.

(Response)
We used same volume (0.2 μl) for forward and reverse primers. Therefore we revised as follows; 0.2 μl and 0.4 μM forward and reverse primers, and 12.3 12.1 μl as the DNA sample.

5. (Table1-4)
Many abbreviations, such as "PPD", "BOP", "SUP" and "ND" are written in the tables. Table legends should be included underneath the table.
According to the reviewer’s suggestion, we added table legends underneath the table 1-4.

6. (Table1-4)
Change "PPD" to "PPD (mm)".

(Response)
We have changed.

7. (Table1)
Males, HC
Change "45" to "5".

(Response)
We have changed.

8. (References)
Please correct the notation of all page numbers as shown below.
BMC Oral Health example reference style:
Article within a journal

(Response)
We have corrected.

9. There are a number of typographical errors that need correcting:
   a. No.8 Change "Roudie RJ" to "Roudier J".
According to the reviewer’s comments, we have corrected.

Reviewer 2 (Dr. Jannet Katz)

Main concerns:

1. The authors showed in 2013, (PLoS ONE 8(8): e71990) the presence of EBV and P. gingivalis DNA in deep periodontal pockets of patients with chronic periodontitis (CP). In the manuscript under review, the authors showed the presence of EBV and P. gingivalis DNA in deep periodontal pocket of peri-implantitis (PI) patients. Thus, it is unclear what new information is being provided in the current manuscript. Assessing differences in the cytokines
that may be present in the deep periodontal pockets of the different groups of people evaluated in the study under review, would have provide novelty. Furthermore, it would have been intriguing to determine if vIL-10 is present in the deep periodontal pockets evaluated, since EBV is known to encode this immunomodulatory cytokine.

Furthermore, the association between herpes viruses and periodontal pathogens like P. gingivalis has been shown in previous publications (appropriately cited by the authors), hence making it more difficult to determine the novelty of the study under review. Moreover, while the authors suggest an association between EBV and P. gingivalis that likely contributes to the etiology and pathogenesis of periodontal disease, they do not show or discuss how EBV promotes the etiology and development of periodontal disease.

(Response)

Thank you very much for your valuable comments. We have previously reported higher prevalence of EBV DNA and P. gingivalis in deeper periodontal pockets of Japanese chronic periodontitis patients. Clinical and pathological conditions of chronic periodontitis and peri-implantitis are quite different. Therefore, in this study, we have shown that P. gingivalis and coexistence of EBV and P. gingivalis were detected significantly higher in the peri-implantitis patients than healthy controls and healthy implant patients. We believe the results in this manuscript are novel.

We are very interested in the levels of cytokines in the deep periodontal pockets of the different groups (periodontally healthy individuals, healthy implant patients and PI patients), therefore we would like to report that in our future studies.

In the Discussion, we described the possibility of how EBV promotes the etiology and development of periodontitis and PI, although the reviewer mentioned the Discussion is highly speculative.

2. EBV is transmitted through saliva and studies have shown that gingival epithelial cells are infected with EBV and may serve as an oral reservoir of latent EBV-infected cells. Moreover, over 90% of the population is infected with EBV and infection of EBV is widespread and not limited to the periodontal foci. Thus, it would be interesting to determine the presence and quantity of EBV in the saliva of healthy periodontal patients, healthy PI patients and PI patients. Would there be a difference in the levels of EBV in these groups? Would a higher viral load occur in PI patients?

(Response)

We thank the reviewer for this insightful suggestion. There were two studies determine the presence and quantity of EBV by PCR using saliva samples from pregnant woman with chronic
periodontitis [19], and PI patients [39]. We would like to determine the presence and quantity of EBV in the saliva samples of healthy periodontal patients, healthy implant patients and PI patients. Therefore, we would like to report the results in the near future.

3. Based on a paper published by one of the authors in 2012 (Biochimie 94, 839e846), the authors suggest in the manuscript under review (Discussion section), that the presence of P. gingivalis reactivated the latent EBV due to the butyric acid produced by P. gingivalis. Butyric acid was shown to be an inhibitor of HDAC, consequently increasing histone acetylation and the transcriptional activity of the BLZF1 gene, which is a marker of active EBV. While activation of EBV by sodium butyrate has been used for many, many years, the novelty of their paper rested on the interesting observation that P. gingivalis via the butyric acid it produces in the supernatant could drive EBV to induce lytic replication determined by the transcriptional activity of the BLZF1 gene. However, all the studies in this published work were done in vitro. In the manuscript under review, the amount of butyric acid in the deep periodontal pockets was not assessed, thus, the suggested reactivation of EBV by P. gingivalis is at best speculative. Assessing the levels of butyric acid in the deep periodontal pockets may be a very difficult task, but the authors could estimate the number of P. gingivalis from the real PCR data obtained in the deep periodontal pockets of the different patients and set up the correspondent P. gingivalis cultures to determine the amount of butyric acid present in the supernatants. The supernatants could then be used to determine if they activate latent EBV in a Daudi (or AKATA) cell line by assessing the transcriptional activity of BLZF, RTA, or gp350 expression.

(Response)

We appreciate this positive comment. Assessing the levels of butyric acid in the deep periodontal pockets could be difficult as reviewer’s mentioned above. Reviewer suggests we could estimate the number of P. gingivalis from the qPCR data obtained in the deep periodontal pockets of the different patients and set up the correspondent P. gingivalis cultures to determine the amount of butyric acid present in the supernatants. We would like to perform the experiment mentioned above in our future study.

Minor concerns:

1. The authors should obtain assistance from a fluent English speaking person to edit their manuscript as there are some unclear statements.

(Response)
We apologize for the quality of written English. We have extensively improved the use of English.

2. The authors mentioned that PI provokes bone destruction with suppuration (SUP), through peri-implant mucositis (PM), but in the Methods section, they stated that PM "was defined as bleeding on gentle probing" and PM is never used again in the manuscript. Therefore, the term peri-implant mucositis (PM) should be altogether eliminated.

(Response)

According to the reviewer’s suggestion, we eliminated the description of peri-implant mucositis (PM).

3. It is puzzling why "healthy sites of PPD" (healthy sites do not have deep periodontal pockets) or better said healthy gingival sulcus was to be less than 3 mm among the healthy controls, but the healthy implant sites were accepted to be less than 4 mm.

(Response)

Thank you very much for your valuable comments. The structure and histology of periodontium and implant are completely different, and generally probing depth of implant is deeper than natural teeth. So, we believe that the diagnostic criteria of periodontally healthy site of PPD (<3 mm) and healthy implant site of PPD (<4 mm) is appropriate.

4. While in general there is room for speculation based on results obtained and published literature, in the manuscript under review, the Discussion is highly speculative without foundation as the only thing that was assessed was the DNA of EBV and P. gingivalis in PI patients and the copy numbers of these were compared with that found in healthy periodontal patients and in healthy PI patients.

(Response)

Thank you very much for your valuable comments. We would like to estimate the number of P. gingivalis from the qPCR data obtained in the deep periodontal pockets of the different patients and set up the correspondent P. gingivalis cultures to determine the amount of butyric acid present in the supernatants as reviewer’s suggested. We would like to perform the experiment mentioned above in our future study.
5. Tables lack footnotes. I assume that ND=Not detected? Table 1 indicates that 45, instead of 5 healthy control males were used.

(Response)

Thank you very much for your comments. We revised Tables.