Author’s response to reviews

Title: A randomized, double-blind, placebo-controlled multicenter study for evaluating the effects of fixed-dose combinations of vitamin C, vitamin E, lysozyme, and carbazochrome on gingival inflammation in chronic periodontitis patients

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Author’s response to reviews:

Dear Editor

We are very grateful to you and the journal’s reviewers for the critical comments and useful suggestions that have helped us to improve our paper. We have taken all these feedbacks into account and submit a revised version of our manuscript.

Referee(s)’ Comments to Author:

Farhan Raza Khan, BDS, MS, MCPS, FCPS (Reviewer 1)
1. Generalized estimation equation analysis should be done

Answer: Thank you for the important comments about statistics. Generalized estimation equation (GEE) analysis was additionally done for the various outcomes (response variables) including GI, PI, PD, CAL and 100 mm VAS with adjustment of confounding factors (age, gender, visit times and treatment groups). The results of GEE analysis was presented in Table 3. In the results, test group showed 2.5 times of GI improvement compared to the control group (odds ratio 2.457, \(p = 0.022\)). In case of VAS, however, there was no significant difference on reduction effect between groups (odds ratio 1.440, \(p = 0.372\)). The results about the GEE was described in the manuscript as follows (also, highlighted in the manuscript);

Abstracts section, line 13-14, page 3

Materials and methods - statistical analysis section, line 3-7, page 10

Results section, line 4-6, page 11

Results section, line 13-14, page 11

Tables section, line 5, page 23 (also, Table 3 was newly added)

2. Better to present data on all standing teeth than Ramfjord teeth

Answer: Thank you for the important comments and we totally agree with the fact that it would be much better to collect data from full mouth examinations. However, it was designed to use Ramfjord teeth in the beginning of the present work to enhance screening and enrollment of the participants at the participating multicenters. In addition, as the participants with chronic incipient periodontitis were enrolled in the study, probing depth and clinical attachment level might show similar range throughout the whole dentition. Even though this design has various limitations, the selected teeth with the standard criteria can be used as representative values. We have emphasized the contents about limitations and controversial opinions on using partial mouth examinations in the manuscript as follows (also, highlighted in the manuscript);
The patients enrolled in the present study included those who had PD values of 4–6 mm at at least one site for a quadrant and were diagnosed with chronic incipient to moderate periodontitis. However, the baseline data on PD in the control and test groups was 2.49 ± 0.39 mm and 2.63 ± 0.47 mm, respectively, of which the severity of periodontal disease might be considered to be in a mild state. The discrepancy in periodontal status may be due to the selected recordings of the Ramfjord teeth (teeth numbers 16, 21, 24, 36, 41, and 44) used in this study. Although a full mouth examination is considered the gold standard, there have been reports showing high agreement between Ramfjord teeth and full mouth periodontal probing that validated the partial recording in an epidemiological study [29, 30]. This technique might be more effective in handling a large sample size, but controversial opinions have demonstrated lower intraclass correlation coefficients for Ramfjord teeth assessments for the percentage of sites over a higher threshold and underestimation of disease prevalence [31]. The partial recording system of selected teeth in the present study might have limited the precise interpretations of the changes in the clinical results.

3. Instead of focusing on GI alone, better to discuss on periodontal parameters such as CAL, PD, BoP etc.

Answer: Thank you for the comments. We have added the discussion about periodontal parameters including PI, PD and CAL along with GI in the manuscript as follows (also, highlighted in the manuscript);
The results in the present study showed significant improvement in the mean change of GI within the first 4 weeks of the test group compared to the control group. The test group showed significant reduction in GI at both 4 weeks and 8 weeks from the baseline, whereas the control group showed no significant difference. Additionally, the test group showed approximately 2.5 times improvement (odds ratio 2.457, p = 0.022) compared to the control group when the confounding factors including age, gender, and visits were adjusted. However, the comparison of GI at each time point failed to show a significant difference between the groups. Other periodontal parameters including PI, PD and CAL did not show any significant differences in the test group when compared to the control group, although PI at 8 weeks and PD at 4 weeks and 8 weeks in the test group showed significant reduction from the baseline value. It can be assumed that these comparable outcomes are due to the SRPs done equally for both groups as the mechanical removal of plaque has effects on the improvement of PI, PD and CAL. Adjunctively supplemented CELC with SRP might have benefits for the reduction of superficial gingival inflammation, but the clinical effect did not reach the soft tissue status around the pocket base to change PD and CAL. Furthermore, it is still difficult to assert CELC’s clinical impact on gingival inflammation with a clear-cut conclusion as to the amount of mean change was very limited. To clarify its clinical efficacy, data from a larger sample size with full mouth examinations and longer study periods should further be obtained. Since the target subjects for adjunctive pharmaceuticals might include the ones with higher disease susceptibility, strict baseline criteria to enroll patients with severe periodontal disease should also be performed.

Jan Kühnisch (Reviewer 2)

The present RCT is investigating the effects of a pharmaceutical composed of vitamin C, vitamin E, lysozyme, and carbazochrome on gingival inflammation of chronic periodontitis patients. It was shown a mild, short-term (8 weeks) effect according to the gingival index. Other periodontal indices showed non-significant differences. There are some suggestions which need to be addressed during revision of the manuscript:

1. The phrase "vitamin C, vitamin E, lysozyme, and carbazochrome …" is repeatedly used in the manuscript. Please modify.

Answer: Thank you for the comments. We have modified the phrase to “CELC” in short and corrected every phrase in the manuscript.
2. The statistical analyses excluded logistic regression analyses so far. It is suggested to analyse
the data in such a way due to the fact that simple comparisons might be misleading and
confounding factors (age, gender) are not considered. Please include an appropriate logistic
regression analyses or refer - at minimum - in the discussion that this statistics was not
performed.

Answer: Thank you for the important comments about statistics. Generalized estimation equation
(GEE) analysis was additionally done for the various outcomes (response variables) including
GI, PI, PD, CAL and 100 mm VAS with adjustment of confounding factors (age, gender, visit
times and treatment groups). The results of GEE analysis was presented in Table 3. In the results,
test group showed 2.5 times of GI improvement compared to the control group (odds ratio 2.457,
p = 0.022). In case of VAS, however, there was no significant difference on reduction effect
between groups (odds ratio 1.440, p = 0.372). The results about the GEE was described in the
manuscript as follows (also, highlighted in the manuscript);

Abstracts section, line 13-14, page 3
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Results section, line 4-6, page 11
Results section, line 13-14, page 11
Tables section, line 5, page 23 (also, Table 3 was newly added)
3. Besides highlighting small statistical significances there is an urgent need to interpret these data from a clinical point of view: With respect the small differences, the data should be interpreted with caution. The effects seem to be true for an 8 week interval and are small. Please revise your conclusion.

Answer: Thank you for the important comments. Despite the statistical significances of GI shown in the test group in 8-week of study period, it is true that we can’t have a clear-cut conclusions on CELC’s efficacy on gingival inflammation from a clinical point of view. It should further be studied in the larger sample size of subjects with full mouth examination data to clarify the CELC’s effect. These contents were added in the manuscript as follows (also, highlighted in the manuscript):

Abstracts section, line 15-17, page 3

Conclusions: Within the study, CELC showed a significant reduction in gingival inflammation compared with a placebo. Other parameters, however, were similar between groups.

Discussion section, line 8, page 13 – line 5, page 14

The results in the present study showed significant improvement in the mean change of GI within the first 4 weeks of the test group compared to the control group. The test group showed significant reduction in GI at both 4 weeks and 8 weeks from the baseline, whereas the control group showed no significant difference. Additionally, the test group showed approximately 2.5 times improvement (odds ratio 2.457, p = 0.022) compared to the control group when the confounding factors including age, gender, and visits were adjusted. However, the comparison of GI at each time point failed to show a significant difference between the groups. Other periodontal parameters including PI, PD and CAL did not show any significant differences in the test group when compared to the control group, although PI at 8 weeks and PD at 4 weeks and 8 weeks in the test group showed significant reduction from the baseline value. It can be assumed that these comparable outcomes are due to the SRPs done equally for both groups as the mechanical removal of plaque has effects on the improvement of PI, PD and CAL. Adjunctively supplemented CELC with SRP might have benefits for the reduction of superficial gingival inflammation, but the clinical effect did not reach the soft tissue status around the pocket base to change PD and CAL. Furthermore, it is still difficult to assert CELC’s clinical impact on gingival inflammation with a clear-cut conclusion as to the amount of mean change was very limited. To clarify its clinical efficacy, data from a larger sample size with full mouth examinations and longer study periods should further be obtained. Since the target subjects for adjunctive pharmaceuticals might include the ones with higher disease susceptibility, strict baseline criteria to enroll patients with severe periodontal disease should also be performed.
Within the limitations of the present study, CELC adjunctively administered SRP exhibited a significant reduction in the index of gingival inflammation in a short-term investigation. Patient’s self-reported gingival discomfort improved in both groups with significant differences at 8 weeks between the two groups. However, changes in other periodontal parameters, including PI, PD and CAL, were similar between the groups. CELC’s effect on the reduction in gingival inflammation should further be clarified with larger sample sizes and clinical data from full mouth examinations. Additionally, studies on adjunctive nutritional intake for compromised populations such as the elderly or comorbidities should be further evaluated.

Conclusions section, line 16 - 19, page 16

Within the limitations of the study, adjunctively supplemented CELC with SRP showed a significant reduction in gingival inflammation compared with a placebo in a short-term investigation. However, other periodontal parameters were similar between groups, and CELC’s clinical efficacy and benefits should further be clarified with a larger sample size.

Editor’s Comments to Author:

1. Please copy-edit your manuscript, we suggest you ask a native English-speaking colleague to help you with this, or use a professional service.

Answer: Thank you for the comments. We used a profession editing service, Nature Research Editing Service (http://bit.ly/NRES-LS), for the manuscript.

2. In addition, we do expect that you will improve the statistical analysis as suggested by reviewer 2, and not just by the addition of further discussion.

Answer: Thank you for the comments. We have done the GEE analysis suggested by two reviewers and added in the manuscript.
We hope that the revised version of our paper is now suitable for publication in BMC Oral Health and we look forward to hearing from you at your earliest convenience.

Sincerely yours,

Jong-Hyuk Chung