Author’s response to reviews

Title: The Effect of Adjuctive Chlorhexidine Mouthrinse on GCF MMP-8 And TIMP-1 Levels in Gingivitis: A Randomized Placebo-Controlled Study

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Re: “The Effect of Adjuctive Chlorhexidine Mouthrinse on GCF MMP-8 and TIMP-1 Levels in Gingivitis: A Randomized Placebo-Controlled Study”

Dear editor,

In reference to your letter dated 20 March 2014 about the reviews of our manuscript, I am sending you our revised manuscript. We have performed our best to correct the sections that were pointed out. We have added our explanations to the comments. We have submitted the amendments to the reviewer at the following pages.

We would very much appreciate the interest you have shown for our manuscript.

Sincerely yours,

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Point-to point Amendments to Reviewer 1

1. Study Design related Problems

Effectiveness of Oral Hygiene Instructions

Although the aim of this study was to evaluate the additional benefit of an antibacterial CHX mouth rinse on clinical and serological parameters in patients with instructed oral hygiene, the data from the placebo group suggest, that the oral hygiene instructions given were basically useless and had very little if any impact on the quality of oral home care as indicated by the level of gingival papillary bleeding or the level of plaque on the tooth at baseline and at week 4.

We thank the reviewer for comments. However we do not agree with the reviewer about the oral hygiene instructions given are useless and have very little effect on the accumulation of plaque and papillary bleeding index. If the whole mouth probing depth (PD), plaque index (PI) and papillary bleeding indexes (PBI) are taken into consideration, it might be observed that both treatment strategies (CHX and placebo mouthrinse) resulted in similar statistically and clinically significant improvements on these parameters at four weeks visit when compared with baseline levels (from 2.16 ± 0.3 to 1.94 ± 0.3 for PD value, from 1.47 ± 0.5 to 0.80 ± 0.5 for PBI, from 3.15 ± 0.6 to 1.69 ± 0.6 for PI in CHX group, from 2.34 ± 0.4 to 2.10 ± 0.4 for PD value, from 1.44 ± 0.7 to 0.96 ± 0.6 for PBI, from 3.15 ± 0.6 to 2.56 ± 0.5 for PI in placebo group) (p < 0.05).

Additionally, CHX group showed lower PI values than the placebo group at study termination (p < 0.05) and the reductions in the PI were significantly greater (p < 0.05) in the CHX group than placebo group at 4th week. We mentioned this information in the manuscript (page 9, under the section named as “Clinical periodontal parameters of whole mouth”), but did not give the data by using a table because we had given those data in our first published paper.1

Secondly, all study participants in the present study were gingivitis patients and they had gingival inflammation at the baseline. All the patients received toothbrushing instructions. However we did not demonstrate to the patients how to make interdental cleaning of the interproximal surfaces of the teeth, because we aimed to evaluate the adjunctive effect of chlorhexidine mouthrinse in addition to daily plaque control performed by toothbrush alone. Sampling sites were chosen among the interproximal sites of the teeth in the present study. Therefore, it is very reasonable that the decrease in PI and PBI scores of sampling sites was not statistically significant at the 4th week compared to baseline although whole mouth plaque and papillary bleeding scores were significantly decreased. Besides, we believe that significantly lower PI values at posterior sampling sites of CHX group at four weeks compared to those sites of placebo group demonstrates the additional effect of CHX on plaque accumulation especially in posterior sites where mechanical plaque control is more difficult than the anterior sites, which is compatible with the knowledge of antiplaque effect of CHX.2-5
References


2. A third experimental group neither receiving any oral hygiene instructions nor any rinsings over the 4 week period would have allowed to quantitate the effect of oral hygiene instructions alone.

The aim of the present study was to evaluate the adjunctive effect of chlorhexidine mouthrinse in addition to daily plaque control performed by toothbrush on clinical and serological parameters, therefore we did not prefer to involve a third experimental group neither receiving any oral hygiene instructions nor rinsing. Furthermore, it is not possible to get ethical approval from the ethical committee in order to compose the suggesting study group by reviewer.

3. Statistical Analysis

In the manuscript a p-value (p<0.05) is given for comparisons which revealed no significant differences between the two experimental groups. The validity of the statement of "no difference" however needs to be verified by the calculated test power.

The present study had a power calculation, which can be seen at the page 8 (under the section “Statistical analysis”).

Furthermore during the statistical analysis a multitude of group comparisons were made. It is not stated in the manuscript whether the level of significance (p<0.05) has been adjusted accordingly for multiple testing.

There were two study groups in the present study; the CHX mouthrinse group
and the placebo mouthrinse group; and clinical parameters and GCF MMP-8 and TIMP-1 levels were evaluated at baseline and 4th week. Intragroup comparisons of the GCF MMP-8 and TIMP-1 levels and the clinical parameters of the study sites between baseline and four weeks were tested by Wilcoxon signed rank test to analyze the significance of changes over time, and the Mann-Whitney test was used to determine significant differences in GCF MMP-8 and TIMP-1 levels and the clinical parameters of the study sites between the CHX and placebo groups. Therefore no adjustment was performed for P value, and the P value for statistical significance was 0.05.

4. The recording of the Papillary Bleeding Index PBI (Saxer & Muehleman) is not explained during its first appearance in the manuscript. As suggested by the reviewer, we corrected this mistake.

5. It is not explained in the manuscript whether ELISA and IFMA were performed using commercial test kits (which kits?). ELISA kit for TIMP-1 is a commercially available test kit, but MMP-8 IFMA is not commercially available and MMP-8 IFMA is not a kit, it is a fluorometric immunoassay (IFMA). IFMA utilizes two monoclonal anti-MMP-8 antibodies and exerts higher accuracy. The antibody identifies the neutrophil- and fibroblast-type MMP-8 isoforms and particularly their active forms in IFMA technique. MMP-8 levels were determined by a time-resolved immunofluorescence assay (IFMA).1

Reference


This information was added to manuscript (please see page 8).

6. A drop-out rate of 38% (37/97) is unusual for a simple mouth rinse trial and should be explained more in detail.

As reviewer mentioned, drop-out rate of the present study is very high. Thirty-seven patients were excluded from the study during clinical trial due to following reasons:

1. 16 out of them excluded from the study because they did not use the mouthrinse regularly. The period of this clinical trial was 4 weeks, which might be considered as a relatively long period of any kind of drug usage and it makes the compliance difficult. We think that high number of drop-outs is caused by this relatively long period of mouthrinse usage.

2. A total of six patients in both group excluded from the study because they discontinued the study. 2 of them moved to another city, and we were not able to connect to the others although we phoned them several times.

3. Eleven patients excluded from the study because they received antibiotics
during the experimental period. Unfortunately, antibiotic usage is very common in Turkey, therefore we had to exclude those patients from the study.

4. Four patients excluded from the study because they had mucosal ulcerations.

We added an explanation for this high drop-out rate to “Discussion” section (please see page 14).

7. The English language of the manuscript is in need for improvement and should be revised by a native speaker.

We did our best to correct grammar mistakes.

Point-to point Amendments to Reviewer 2

As emphasized by the authors, matrix metalloproteinases play an important role in periodontal tissue destruction. The manuscript describes the effect of chlorhexidine (gold standard antimicrobial agent) on GCF levels of matrix metalloproteinase MMP-8 and tissue inhibitor of matrix metalloproteinases TIMP-1 in gingivitis patients. It should be highlighted that this study is a carefully designed placebo-controlled clinical study. In the pre-screening phase 215 patients were assessed for eligibility for the study. In the end 50 gingivitis patients meeting inclusion criteria were assigned to CHX or placebo group. The methods and aims are well defined and described. The tables and figures are self-explanatory and appropriately complement the results section. The research found no additional benefit of 4-week adjunctive CHX treatment on GCF MMP-8 and TIMP-1 levels in gingivitis patients. On the other hand, it was shown that 4-week daily plaque control increased GCF TIMP-1 levels regardless of CHX treatment. The limitations of the study are objectively summarized in the discussion.

To conclude, the manuscript presents novel and original data and the conclusions drawn are adequate. The topic is of interest in the field of periodontal diseases. I have no further comments and suggestions how to improve the manuscript. I would recommend to accept the manuscript for publication.

We thank to reviewer for his comments.