Author’s response to reviews

Title: Evaluation of serum zinc levels in patients with recurrent aphthous stomatitis (RAS)

Authors:

Zuzanna Ślebioda (zuzia_slebioda@o2.pl)
Ewa Krawiecka (krawiecka.ewa@gmail.com)
Elżbieta Szponar (stomzach@ump.edu.pl)
Barbara Dorocka-Bobkowska (b.dorocka@gmail.com)

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Author’s response to reviews:

Dear Editor,

We appreciate the time and efforts by the editor and referees in reviewing this manuscript. We have addressed all issues indicated in the review report, and believed that the revised version can meet the journal publication requirements.

Response to Comments from Reviewer #1

Comment 1:

"Firstly the fundamental research hypothesis is weak as written. My recommendation is to expand the research hypothesis for a relationship between Zinc and RAS. Otherwise this may appear as more of a "fishing" expedition. Equally in the introduction, there is no mention of potential sub-groups MiRAS, MaRAS and HeRAS and how these might relate to serum Zinc. As the work looks at sub-groups some explanation should be made in the introduction together with more commentary on the proposed hypothetical Zinc relationship with RAS"

Response:

We greatly appreciate the reviewer’s suggestions. As indicated, we have expanded the explanation of potential role of zinc in the development of RAS in the Background section (page 4-5; lines 101-119). Although in the original version of our paper in the Background section we had actually described the classification criteria for all the study subgroups- MiRAS, MaRAS and HeRAS (and we had also illustrated this stratification in our original Table 1.), to fulfill the Reviewer’s suggestion more detailed description of the study subgroups was added in the Background section (page 3; lines 73-80). In the same time, responding to the Reviewer #3 comment on Tables, we deleted this original Table 1 (presenting the RAS classification criteria).
Finally the classification is presented in a descriptive form on page 3; lines 73-80, as stated above.

Comment 2:

"Secondly there is no explanation of the statistical hypothesis or justification for a sample size and variability expected in the research. The reader has no understanding why 75 subjects were chosen or whether it is a convenience sample. However statistical comparisons have been undertaken and therefore more explanation of the statistical plan prior to the research should be included. If this is a pilot/preliminary study then 75 seems too large. If there is a basis for a sample size of 75, based on prior research, the analytical technique proposed, its sensitivity and specificity then this fits as part of the proposed research hypothesis".

Response:

Thanks for the comment. In the study design the 2-year period of the observation was planned. In this time we included almost all the RAS patients attending our Department, also those who responded to an invitational letter to participate in the study. We managed to recruit 75 RAS patients who fulfilled the study qualification criteria. The statistical methods were suitably adjusted for the comparisons on the described samples of patients.

Several papers published on zinc status in oral mucosa and skin diseases (including RAS) were conducted on a similar or considerably smaller sample sizes (see References: 7, 13, 14, 17, 19 and 23).

Comment 3:

"Thirdly assuming the statistical conclusions are valid and that there is no relationship to serum zinc levels the discussion needs to tie back to the first two points. It may be useful to speculate on further research hypothesis / directions."

Response:

We have expanded the discussion of our study results taking into consideration the study subgroups and the sample size (Conclusions, pages 9-10; lines 253-264).

Comment 4:

"Some commentary should be added that none of the patients had active RAS at the time of study".

Response:
Only the patients with RAS, who reported the disease in the history and presented the active lesions during the examination, were enrolled in the present study. It is described in Material and Methods section (page 5; lines 132-134).

Comment 5:
"As a general comment while serum Zinc has been analysed for each subject, an analysis of salivary zinc (and comparison) may have added to the research and make it even more relevant to Oral Health Research".

Response:
Thank You for this suggestion. Definitely the evaluation of salivary zinc levels would enrich the results of the study. We will try to include this analysis in our future research.

Response to Comments from Reviewer #2

Comment 1:
"In the abstract, it is stated that the control group was age and sex-matched. However, the F:M ratio in the control group is 53:19 as compared to 46:29 in the RAS group. Therefore, the claim of being gender-matched should be removed and this should be acknowledged as a limitation of the study in the Discussion."

Response:
We appreciate the suggestion. As indicated, we have removed the false information on gender-matched subjects from the Abstract. We have also added the information on the study limitations in the Conclusions section (pages 9-10; lines 261-264).

Comment 2:
"Additional information should be provided on the control group. How was it determined that they were "generally healthy"? Was a full medical history taken? Were they questioned about past history of oral ulceration?"

Response:
Thanks for the comment. We have introduced more detailed and clear description of the classification criteria used for both: study and control groups in the Material and Methods section (page 5; lines: 135-142).
Comment 3:

"As nutritional deficiencies can vary by region, it should be specifically stated in the manuscript which region of which country the RAS patients and controls were recruited from."

Response:

As suggested, we have defined more precisely the origin of the study participants in Material and Methods section (page 5; line 122).

Comment 4:

"The citation numbers provided along with several references in the text do not match with the respective reference number in the bibliography. This should be carefully checked for all references and corrected as needed".

Response:

Thanks for the suggestion. We have carefully checked and corrected the reference numbers.

Response to Comments from Reviewer #3

Comment 1:

"A description of study design is lacking. This paper seems to be a cross-sectional study".

Response:

We appreciate the suggestion. We have defined the study design more precisely in Material and Methods section (page 5; line 121).

Comment 2:

"Give the eligibility criteria for both groups, and the sources and methods of selection of all participants.

Give diagnostic criteria of RAS"

Response:

As suggested, we have described more clearly the eligibility criteria for both groups together with the methods and sources of the recruitment (see: Material and Methods section, page 5; lines 121-128).
Diagnostic criteria for RAS in general as for the study RAS subgroups are presented in Material and Methods section (page 5; lines: 135-142). Clinical features of RAS are also discussed in Background section (page 3; lines 73-80).

Comment 3:
"Explain how the study size was arrived at"

Response:
(See: Response to the Reviewer’s #2 second comment).

In the study design the 2-year period of the observation was planned. In this time we included almost all the RAS patients attending our Department, also these who responded to an invitational letter to participate in the study. We managed to recruit 75 RAS patients who fulfilled the study qualification criteria at that time. The statistical methods were suitably adjusted for the comparisons on the described samples of patients.

Several papers published on zinc status in oral mucosa and skin diseases (including RAS) were conducted on a similar or considerably smaller sample sizes (see References: 7, 13, 14, 17, 19 and 23).

Comment 4:
"Add the number of examiners
Give the agreement between examiners, intra and inter”.

Response:
We have now defined clearly the number of the examiners (which is 1) in the text (Material and Methods section (page 5; line 133).

Comment 5:
"Describe the rationality of each statistical test used. For example: "We divided the zinc concentration in normal/abnormal (detail) and compare with the presence or absence of RAS. We assess the independence of observations with the chi-square test."

Response:
According to the Reviewer’s instructions we have described the rationality of statistical tests in Material and Methods section, page 5, lines: 141-147.
Comment 6:

"The table are not useful. Instead use

Table 1. Baseline measures, comparing demographic data for both groups, with p values

Table 2. Compare the mean of ZSL (with confidence intervals) between the groups.

Table 3. Add a graph comparing the mean ZSL (with confidence intervals) for the sub diagnostic categories."

Response:

In accordance with the Reviewer’s suggestion, we have rearranged the Tables. Original Tables were replaced with: Table 1., presenting baseline measures and demographic data in the study and control group and Table 2., showing the mean ZSL with CI. We also added a graph comparing the mean ZSL with CI in sub-groups as indicated in the comment.

Comment 7:

"Page 8 line 208 .The study design cannot confirm or deny the RAS as trigger factor. A longitudinal study is required for this aim. Delete and discuss the results taking in consideration the limitations of the current design".

Response:

As suggested we have deleted the sentence on RAS as a trigger factor. The limitations of the present study design were discussed in the Conclusions section, page 9-10; lines 253-264.