Reviewer's report

**Title:** Influence of type 2 diabetes on local production of inflammatory molecules in adults with and without chronic periodontitis: a cross-sectional study

**Version:** 1

**Date:** 22 April 2015

**Reviewer:** Poliana Duarte

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Major Compulsory Revisions

This study evaluated the levels of 27 biomarkers in the GCF of patients with type 2 DM and CP, only CP and only type 2 DM. Considering the high prevalence of DM and CP as well as the need of a better understanding of the molecular mechanisms involved in the pathogenesis of DM-related periodontitis, the topic of study is important. However, the paper presents major concerns, especially those related to the methods and interpretation of the results, as follows:

**Abstract**

Methods: “The material comprised…” It is better “The study population comprised…”

Results: This section should be extensively revised, considering that some biomarkers were statistically similar between groups, as represented by the letters “a,b” in tables (please see the commentary in the results section). According to tables, IL-1# IL-1#, GM-CSF and IL-8 did not differ among groups. Please, revise this and other possible misinterpretation. In addition, this section should not present the tendencies and the non-significant negative correlation between the anti-inflammatory cytokines and the glycated hemoglobin levels. Please, focus on the statistically significant results after adjustments for confounders in this section.

**Conclusion:** This section is not coherent with the results that demonstrated that DM and CP, together and individually, may affect the GCF levels of biomarkers. Please, revise.

**Background**

First paragraph: The worldwide data of DM epidemiology should be presented instead of the Sudan data.

Last paragraph: The hypothesis should not be presented, as none inference was made regarding this hypothesis throughout the text.

**Methods**

One of the major drawbacks of this paper is the criterion used to diagnosis CP (i.e. at least two sites with bleeding pockets ≥ 4mm). According to the criterion determined by the authors, CP patients may have only minor or even no
periodontitis, as clinical attachment loss was not taken into consideration. Pockets without attachment loss may be attributed to excessive gingival overgrowth due to altered passive eruption or due to excessive gingival inflammation.

Calibration details were neglected.

Another the major drawback of this paper is the absence of information regarding the characteristics of the sites selected for GCF sampling. If a patient with CP has attended to the minimum criterion (i.e. only two sites with bleeding pockets ≥4mm), it means that of the four sites selected for GCF sampling in this patient, two had probing depth < 4mm. It seems that sites with totally different characteristics were pooled. Standardizing the characteristic of the selected sites among groups may exclude the possible interference of disease severity on the levels of biomarkers. Were the increased/decreased levels of biomarkers a direct consequence of the diabetic or periodontal status?

The authors did not use a device to measure volume of GCF but measured the total amount of protein in each sample. Although the authors provide adjustment for the total protein in the GLM analysis, it is recommended to express each biomarker level as a proportion relative to the total protein level. The total protein should be used to normalize the data.

Another important concern is the inclusion of smokers without statistical adjustments for this important confounder. Smoking is a well-recognized risk factor for periodontal diseases that has direct effects on the biomarker levels. The percentage of smokers in the CP group is higher when compared to the other groups.

Results

Table 1: It is pocket depth related to the full-mouth? Mean pocket depth of the DM group was not presented. Clinical parameters of the sampled sites were not presented.

This section should be revised in order to describe the actual differences among groups according to the statistical analysis. For example: “The DM+CP group had the highest levels of IL-1#, IL-8, MIP-1# and GM-CSF,...” IL-1#, GM-CSF and IL-8 did not differ among groups. MIP-1# description is not coherent with table 2. “The CP group had the highest levels of IL-4, IL-9, IL-17, TNF-#, MCP-1, MIP-1#, RANTES, FGF, PDGF, and INF-#,...” The authors should be aware that the IL-4, TNF-#, IL-17 ... levels in CP group did not differ from DM group. IL-9 did not differ among groups. “The DM group had the highest levels of IL-2, IL-6, IL-7, IL-10, IL-12, IP-10, VEGF and G-CSF...” Please, revise according to the abovementioned comments.

“Group analysis of the detected inflammatory...” The presentation of “tendencies” should be avoided.

In addition, it is important to mention that the correlation between pro-inflammatory cytokines and the glycated hemoglobin is slight (correlation coefficient = 0.27).
Discussion
The discussion section is too speculative and should be revised according to the actual statistical differences observed among groups.
First paragraph is no necessary.

IL-6 was included as a Th-2 protein in the Th1/Th2 ratio. However, the references used in the discussion section for the statement that this cytokine is multifunctional presenting pro- and anti-inflammatory activities are not appropriated. Please, revise.

“In the present study, the level of IL-1# was highest in the DM+CP group.” This statement is not coherent with the tables.

“This is in accordance with a study which reported a significant positive correlation between IL-1# levels in GCF and HbA1c [34]. The mentioned study (34) only studied the IL-1#.” Therefore, it is not in accordance with the present study.

“This process might be disrupted in patients having both T2DM and periodontitis, as indicated by the lower levels of IL-6 in the DM+CP group than in the DM and CP groups.” IL-6 levels were similar between CP and DM groups.

Conclusion is too speculative and not coherent with the findings of the study. This section is not coherent with the results that demonstrated that DM and CP, together and individually, may affect the GCF levels of biomarkers. Please, revise.

Level of interest: An article of importance in its field

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.