

Dear Editor and Reviewers,

Much thanks for frank evaluation of our work, and for the time you have spent when reviewing our manuscript. We agree with nearly all of your remarks and have made appropriate modifications of the primary version (see "change.pdf" file attached in the supplementary section).

The answers to questions from reviewers are presented below. We offer numbering for convenience.

Reviewer 1

Good job, with solid results. Well done!

Thank you for the great assessment of our work!

Reviewer 2

The features of gingival sulcus microbiome in patients with chronic periodontitis associated with type 2 diabetes mellitus. The study requires profound changes for the consideration of being published.

Abstract

Methods: It is necessary to describe periodontal pathology according to the latest classification, published in 2018, and to describe whether patients with diabetes are compensated or decompensated. The form and characteristics of the sites that are included in the study must be indicated. Also indicate the number of men and women, with the average age of the subjects.

We fixed it. We were described periodontal pathology according to the latest classification, published in 2018. According to the classification, generalized chronic periodontitis in both periodontitis groups (CP, CPT2DM) patients was moderate (stage II). Each group had approximately equal numbers of males (22 in total) and females (24 in total) with an average age of 53.9 ± 7.3 and 54.3 ± 7.2 years old, respectively. We added this correction in the article text.

Results: According to the previous point, the results must be described according to the variable of compensated or decompensated diabetic subjects.

Yes, it's true. In article text no patient description according to the variable of compensated or decompensated diabetic subjects. It was fixed. All patients have a compensated diabetes. We added this correction in the article text.

Conclusions: It is not possible to indicate that it is related to immune or glycemic alterations, if there are no results of that in the study.

We corrected our conclusions according to the reviewer's comment.

Background:

It is necessary for the authors to make greater reference to the relationship they describe in their fundamentals between periodontitis and controlled diabetes, or between periodontitis and patients with diabetes with poor metabolic control. In the case of decompensated patients, describe what is related to the blood glucose and glycated hemoglobin values.

All patients have compensated diabetes. We corrected the article text according to the reviewer comment.

Material and Methods

Subject population and study design

Describe the characteristics of diabetic patients. Indicate the duration of the study. Indicate the criteria used for the sample design, both in the antecedents for the determination of the number of subjects as well as the process for the selection of patients.

We corrected the article text according to the reviewer's comment.

Diagnostic and inclusion criteria: The diagnosis of periodontal pathology should be made using the criteria of the classification published in 2018. Explain whether the criteria for including patients in the study has been used in other publications, otherwise its use should be made explicit.

We corrected the inclusion criteria according to the reviewer's comment. We were described periodontal pathology according to the latest classification, published in 2018. We added this correction in the article text.

In the present study, it is indicated that patients with HBA1c values of 6.5% to 11.3% have been included. This means that the patient with a value of 11.3% has poor metabolic control of his glycemia. This variable is very important and the results and analysis must be presented taking into account this variable, that is, compensated and decompensated diabetic patients.

Yes, it's true, patients with HBA1c values of 6.5% to 11.3% have been included. However, it's a mistake. One patient has a HBA1c level 11.3%, but he was not included in the study. The clinical data and the statistical analysis has been checked and corrected. Patients with compensated type 2 diabetes only were added to the study. Patients with uncompensated type II diabetes and / or severe forms of this pathology were excluded from the study. We added this correction in the article text.

The reason for the use of CPITN should be specified and the complete periodontal examination should not be used, determining the level of clinical insertion.

This indicator was removed from the study. Dental indexes were corrected according to the 2018 classification.

The states and degrees of periodontitis must be described, according to the 2018 classification, and these degrees of severity must be associated with good or bad control of diabetes.

Dental indexes were corrected according to the 2018 classification. Patients with compensated type 2 diabetes only were added to the study.

Collection and sequencing of plaque samples: It is necessary to describe how to proceed to the selection of the sites in which the sample is to be taken, how many sites are to be selected, what are the criteria for making that selection.

The material and methods section were corrected according to the reviewer comment. The study groups contained approximately equal numbers of non-smoking males and females aged 41-65 years. Periodontitis patients had stage II (moderate periodontitis) with a generalized lesion, probing pocket depths of 3-4 mm, loss of bone tissue around the teeth no more than 1/3 of the root length, and virtually no tooth loss associated with periodontitis. Patients with type 2 diabetes were in remission. We added this correction in the article text.

Results

The sample is not homogeneous since it has significant differences in the body weight of the subjects, in such a way as the authors decide that the results are not explained by overweight and not by the presence or absence of diabetes?

Yes, indeed the groups are not BMI equal. It was difficult for us to equalize the BMI groups because our diabetic patients had higher values. This is a significant limitation of this study. We added this correction in the article text.

Discussion and conclusions

The present reviewer cannot review the discussion and conclusions, to the extent that the previous observations are not considered

No comments.

Reviewer 3

General Comments:

This article entitled, "The features of gingival sulcus microbiome in patients with chronic periodontitis associated with type 2 diabetes mellitus" made a comparative analysis to describe features of subgingival microbiome in patients with chronic periodontitis associated with type 2 diabetes mellitus. Although this topic has been investigated before in the literature, the results obtained in this research is valuable for publication to compare with previous literature. Therefore, this article is scientifically valid and technically sound. I believe that this manuscript will be suitable for publication after a major revision.

Abstract

1. The conclusion is made with some general comments. Please consider re-writing it according to the data collected.

Yes, it's true. The conclusion was re-written according to reviewer comments.

2. Keyword 16S rRNA gene sequencing does not match with MeSH Terms (Medical Subject Headings). Please select another keyword using MeSH Terms.

It has been fixed.

Introduction

3. The authors provided information about Periodontitis and Periodontitis associated with T2DM. However the authors did not give any information about Diabetes only related changes of oral and gingival sulcus related microbiome in Introduction. Please give more literature-based information in Introduction.

4. "Chronic periodontitis is also common worldwide. Its prevalence among middle-aged adults is approximately 80% [11]..." This reference does not match with the information given here. Please change the reference.

5. "In patients with T2DM, chronic periodontitis usually progresses rapidly and has a complicated course [19]." This reference does not match with the information given here. Please change the reference.

7. "It has been shown that the oral microbiome is a reservoir of persistent human infections [15] and is involved in various pathologies [16, 17], including diabetes mellitus [18]. In patients with T2DM, chronic periodontitis usually progresses rapidly and has a complicated course [19]. A recent study using a mouse model showed that diabetes increases the pathogenicity of the oral microbiome through a mechanism regulated by the pro-inflammatory cytokine IL-17. Dysregulation of the immune response in diabetes leads to dysbiosis of the subgingival microbiome, which contributes to the development of periodontitis in the host [20]." The information given in this paragraph is so dispersed and confusing. Please consider re-writing it. Also, kindly give more literature-based and detailed information about how diabetes related changes in oral and gingival sulcus-related microbial changes affects the immune system and how it contributes to the development of periodontitis in the host.

8. Furthermore, "A recent study using a mouse model showed that diabetes increases the pathogenicity of the oral microbiome through a mechanism regulated by the pro-inflammatory cytokine IL-17." Consider changing this statement or adding more information, because there are lots of pathways like AGEs, cytokines, and mediators and microstructural changes etc. in diabetes related immune-inflammatory changes in Periodontitis.

9. "Previous studies show that the subgingival microbiome plays a key role in periodontitis pathogenesis. There were clear differences in the composition of the subgingival microbiome between the healthy state and periodontitis state [21, 22, 23, 24, 25]. At the same time, there are only few publications focused on features of subgingival microbiota composition in T2DM patients [9, 26, 25] [Casarin et al., 2013; Zhou et al., 2013; Ganesan et al., 2017; Shi et al., 2019]." Please give references in

numerical order. Furthermore, kindly search for an additional similar mistake all over the manuscript and correct it/them accordingly.

10. “Thus, the present study aimed to conduct a comparative analysis to describe more features of subgingival microbiome in patients with chronic periodontitis associated with type 2 diabetes mellitus (CPT2DM).” Please give more detail about what is new in this article compared to previous research.

Yes it's true. All reviewers comments are relevant. We recognized the introduction as unsuccessful. We completely rewrote the introduction. In our opinion, now it is more consistent with the article content.

Material and Methods

“Patients having a mild and severe course of the pathology were excluded from the study; i.e., the course of the pathology under study was moderate in all patients.” Please explain what you mean here.

All T2DM patients had a disease duration of 3 to 7 years with its moderately compensated course, a blood glucose level below 7.8 mmol / L and a glycated hemoglobin level below 8%. We added this correction in the article text.

“All the examined subjects were advised to refrain from chemical or mechanical oral hygiene for 24 hours.” Please explain if you restricted the subjects from oral hygiene? And if you did so, please provide a reference which did the same. In my opinion, it is an unusual approach and it needs to be discussed.

It's our mistake. We added an irrelevant description of the sampling procedure. Biological samples were taken from patients in the morning before hygiene procedures and eating. Approximately 12-15 hours have passed since the last hygiene procedures. We added this correction in the article text.

“Patients were diagnosed with chronic periodontitis according to the clinical and radiological data in compliance with the Patient Management Protocol ”Periodontitis” elaborated at the A.I. Evdokimov Moscow State University of Medicine and Dentistry (Ministry of Health of the Russian Federation) and the Central Research Institute of Dental and Maxillofacial Surgery Dentistry (Ministry of Health of the Russian Federation) in 2012.” Which criteria and classification did you use for diagnosis of Periodontitis? Also, please provide a reference here.

All T2DM patients had a disease duration of 3 to 7 years with its moderately compensated course, a blood glucose level below 7.8 mmol / L and a glycated hemoglobin level below 8%. The diagnostic criteria description was added to the article text.

“The contents of the periodontal pocket in patients with chronic periodontitis and the contents of the gingival sulcus in healthy subjects were the study material.” What do you mean here? Why didn't you add T2DM patients in here?

Patients with T2DM were also involved. We have changed the article text according to the reviewer comment.

“The material was sampled from four spots within the gingival sulcus using sterile paper endodontic posts (# 30)...” Please provide a reference here. Also, please give more information about how you selected a tooth for sampling. Did you take a sample from a tooth or did you selected multiple teeth for sampling and pooled the samples?

The samples were taken in the morning on an empty stomach (from 9.00 to 11.00) before using a toothbrush and other hygiene products. The biological material was sampled from four spots of the periodontal pockets / sulcus at the level of the second molars [Beck et al., 2018] using sterile paper endodontic posts (# 25) [Baymiev et al., 2017], which were placed together in a test tube containing 0.2 ml of sterile physiological saline solution and shaken. We added this correction in the article text.

Furthermore, give more detail about the sampling procedure and provide a reference. “(https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).” There is no need to provide a http link, Please provide more information about brands and production places of materials as you previously did.

It was fixed.

Furthermore, there are too many references given in Materials and Methods. Kindly consider reducing the count of references given here.

It was fixed.

Statistical Analysis

Please provide information about a power analysis.

Information about a power analysis was added to the article text.

Results

Please add a table showing results of Periodontal indices group by group and give results accordingly. Furthermore, please give information about healthy control groups. Is it periodontally healthy or gingivitis? Please provide p values here.

We have changed the article text according to the reviewer comment.

Discussion

“Chronic periodontitis and type 2 diabetes mellitus are widespread multifactorial diseases. These diseases are interconnected.” Please provide a reference here.

It was fixed.

“No significant differences between the two periodontitis groups in any dental index have been revealed.” Please add a table showing results of Periodontal indices group by group and discuss results accordingly.

We added novel analysis according to 2018 Classification. The table showing results of Periodontal indices group by group was added in the article text.

“High bacterial richness in the oral microbiome is significantly associated with poor oral health, including the presence of decayed teeth, periodontitis, and poor oral hygiene [58].” This reference is not suitable here because the authors state that there is no difference regarding periodontal indices between periodontitis groups. Please discuss more deeply why Alpha diversity was increased in the CPT2DM group compared to the Control and CP groups.

We declare that there is a difference in this indicator between the experimental groups and we consider this link to be relevant.

Furthermore, please give information about why you didn't include patients with T2DM as a control group and what are the disadvantages by excluding it. Please discuss accordingly.

Yes it's true. In our study, there is no group of patients with type 2 diabetes mellitus, but without periodontitis. During the entire observation period, we met only three people in whom diabetes mellitus was not accompanied by chronic periodontitis. We considered this group to be small for inclusion in the study.

Please give more detailed information about what is new in this article compared to previous research. This is the critical part of the discussion. Kindly, compare the studies point by point to clarify reasons for different results in the literature.

it was fixed.

These findings are consistent with the results of previous studies using 16S rRNA gene sequencing of subgingival bacterial communities [Griffen et al., 2012]. Please give this reference in numeric.

it was fixed.

“The association of these bacteria with disease severity was noted. *P. intermedia* is associated with more severe forms of periodontitis, while ...” Please give bacteria names in Italics.

it was fixed.

Additionally, please discuss and address any potential limitations of the present study.

This was added to the article text.