Journal of Oral Biology and Craniofacial Research 13 (2023) 500–505

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Efficacy of nonsurgical periodontal therapy affecting salivary biomarkers in non-diabetic and type 2 diabetic periodontitis patients. An observational study

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ARTICLE INFO

Keywords:
Saliva
Salivary biomarkers
Diabetes mellitus
Periodontitis
Periodontal therapy

ABSTRACT

Objectives: To assess the effects of non-surgical periodontal therapy on salivary biomarkers in non-diabetic and type 2 diabetic periodontitis patients and to investigate if saliva may be used to monitor glucose levels in type 2 diabetes.

Material and methods: 250 participants with chronic generalized periodontitis aged 35–70 years were divided into two groups: test group with type 2 diabetes (125 subjects with 64 males & 61 females) and control group with non-diabetic (125 subjects with 83 males & 42 females). Participants received non-surgical periodontal treatment. Pre-NSPT and 6-week post-NSPT saliva glucose, amylase, total protein, and C-reactive protein (CRP) levels were measured. Intergroup correlations were assessed using Karl Pearson's correlation coefficient and paired *t*-test.

Results: Non-surgical periodontal therapy significantly decreased CRP ($p < 0.05$) in diabetics and non-diabetics. CRP mean values changes from baseline 1.79 to post op 1.5 and baseline 1.5 to post-op 1.24 in males and females of test group, respectively. In control group males and females, mean values change from baseline 1.48 to post-op 1.42 and 1.499 to 1.40. Other parameters Glucose, amylase & total protein showed improvement in the level, but statistically non-significant ($p > 0.05$). Salivary glucose levels corresponded favorably with HbA1C levels.

Conclusion: In individuals with type 2 diabetes and non-diabetic generalized chronic periodontitis, non-surgical periodontal therapy may play a role in lowering the level of significant salivary biomarkers. Saliva can be utilized as a non-invasive approach for monitoring glucose levels in people with type 2 diabetes and chronic periodontitis.

1. Introduction

Diabetes is a major cause of death worldwide due to a lack of adequate diagnosis and treatment; more than half of people with diabetes are undiagnosed, particularly those with type 2 diabetes.[1] Diabetes complications and morbidity increase exponentially if not diagnosed early.[2–4] In India, approximately 57.2 million diabetics will be identified by 2025.[5] In addition, it is well understood that glucose is a nutrient for candida microorganisms.[6] Consequences of these elevated salivary glucose levels in diabetes can be hypothesized. Candida, dental caries, gingivitis, periodontal disease, increased risk of infection, burning mouth, dental abscesses, fungal infections, taste impairment, and poor wound healing are some of the oral diseases and conditions that can be brought on by high salivary glucose levels.[7]

Diabetes can now only be diagnosed by measuring glucose levels in the blood. These procedures are both physically and psychologically

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https://doi.org/10.1016/j.jobcr.2023.05.012
Received 10 November 2022; Received in revised form 24 March 2023; Accepted 27 May 2023
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invasive. Consequently, a technique that is non-invasive, straightforward, and painless, such as salivary glucose measurement, is essential. The entire saliva contains locally produced chemicals and serum components that can be used to diagnose and comprehend a number of systemic diseases and oral symptoms.[8]

It would appear that glucose, amylase, and total proteins are the salivary parameters that are most closely related to the oral environment[9] in patients who have type 2 diabetes. Glucose is a small molecule that travels through the membranes of blood vessels, first to gingival fluid and then to saliva via the gingival sulcus.[10] This process occurs in blood plasma. In patients with type 2 diabetes, an increase in blood glucose levels can result in an increase in salivary glucose levels, which can result in a breakdown of homeostasis and an increased vulnerability to illness in the oral cavity.[11]

C-reactive protein (CRP) is one of several markers of inflammation in saliva that is elevated in diabetics. The liver-produced pentameric protein CRP has emerged as the "golden marker for inflammation." It is thought to be a non-immunoglobin protein composed of five identical subunits. It belongs to the pentraxin protein family. The CRP is named after the fact that it reacts with streptococcus pneumonia capsule polysaccharides. It is an acute phase response protein that is significantly elevated in inflammatory and infectious diseases of the body or oral cavity. Although there are several studies on the relationship between hs-CRP and diabetes mellitus in different age groups in the current literature, they are limited. As a result, the current study focuses on the relationship between CRP and diabetes mellitus in different age groups.[12,13]

The use of saliva as a diagnostic technique is gaining momentum. Saliva is viewed as a valuable diagnostic tool for the future due to its non-invasive nature, the absence of the necessity for a qualified technician during collection, and the existence of sensitive biomarkers.[14] Hence, the current investigation compares the responses to periodontal therapy in people with diabetes and those without diabetes by measuring salivary glucose, salivary Amylase, C reactive protein, and total protein levels.

This study aims to evaluate the impact of non-surgical periodontal therapy on the level of relevant salivary biomarkers (Glucose, amylase, total protein and CRP) in the saliva of both non-diabetic and type 2 diabetic periodontitis patients; and concomitantly to determine, if saliva can be used as a non-invasive tool to monitor glucose level in Type 2 diabetes**.**

The researchers hypothesized that after non-surgical periodontal therapy, there is no significant difference in salivary biomarker levels between diabetic and non-diabetic patients.

2. Material and methods

To achieve the objective of the study, the researchers designed and conducted a randomized controlled clinical trial at the Department of Periodontology. The trial was sanctioned by an institutional review board and a local ethics committee, and the research adhered to the Helsinki criteria. Enrolled were all patients who strictly adhered to the implementation recommendations. Between September 2013 and September 2018, the research population comprised all patients who presented for periodontitis evaluation and treatment.

To be included in the study sample, patients had to fulfill the following inclusion criteria:

Group I: Test group.

1. Individuals aged 35 to 70 were diagnosed with chronic periodontitis and periodontal parameters,[15] and those with chronic periodontitis were included in the study.
2. Participants must have type 2 diabetes and a HbA1c of 7.0% or above, as glycated haemoglobin levels >7% are an appropriate aim for patient-centered drug for the management of diabetes mellitus.[16]
3. Presence of a number of teeth more than 10 per dental arch, excluding third molars.
4. No modification in diabetic medication or intake of antimicrobials in the last three months before or during the study.
5. Probing depth more than or equal to 5 mm, with attachment loss more than or equal to 3mm with radiographic bone loss.

Group II: Control group.

1. Patients with chronic periodontitis with an age range of 35–70.
2. Probing depth more than or equal to 5mm.
3. Attachment loss of more than or equal to 3mm.
4. Radiographic evidence of bone loss at more than 30% of the sites examined.
5. Non-diabetic patients, patients with HbA1c levels lesser than 6.0%.
6. Presence of number of teeth more than 10 per dental arch, excluding third molars. The presence of at least a total of 20 teeth is required so that the minimum total surface area of dental periodontal surface is required for systemic effects to result from periodontitis

Patients were excluded as study subjects if.

1. Patients affected with any systemic conditions known to exert effects of any kind in the study or on the assessed parameters.
2. Current Smokers and recent former smokers (Effect CRP levels).
3. Pregnant and lactating females.
4. Any form of periodontal therapy in the previous 6 months.
5. Use of any antimicrobials in the last three months.
6. Any other infections (e.g., Common cold, influenza, ENT infections, etc.) may affect any change in the considered study parameters.
7. Patients undergoing or have undergone radiation therapy in the recent past.
8. Long-standing infections of any kind.
9. Patients on chemotherapy

2.1. Study sampling

The patient sample size was calculated using:

$$n = \frac{N}{1 + N(e)^2}$$

Where N is the Population size and e is the level of precision.

The estimated sample size was calculated to be 125.4 for each group.

By flipping a coin, the participants were assigned to test group (n = 125) diabetic group and control group (n = 125) non-diabetic group.

2.1.1. Methods of collection of the data

Total of 250 patients were included in this study and they were divided into 2 groups:

Test Group - 125 patients with chronic periodontitis patients with type II –Diabetes.

Control Group - 125 patients with chronic periodontitis patients without Diabetes.

All patients had a diagnostic work-up that included pocket depth measurement at 6 probing sites, 3 buccal (mesiobuccal, mid buccal, and distobuccal) and 3 lingual (mesiolingual, mid-lingual, and distolingual) sites with a UNC-15 periodontal probe, Clinical attachment level (CAL), one panoramic radiograph, and a full mouth set of 18 parallel technique intraoral-periapical radiographs for radiographic alveolar bone loss reading All participants underwent standard laboratory investigations. All subjects provided written informed consent. To control for bias in the study, all subjects had a clinical periodontal examination performed by a single examiner who manually recorded all variables.

2.2. Non-surgical periodontal therapy

Oral prophylaxis was administered to all quadrants (diabetic group and non-diabetic group). First, scaling was done with a piezoelectric ultrasonic scaler (Woodpecker UDS P Led ultrasonic scaler, Woodpecker) and manual instruments as needed, taking about an hour in each quadrant.

2.3. Blood sample collection

The participants were instructed to sit comfortably on chairs with one arm extended straight from the shoulder. A tourniquet was placed 1.5–2 inches above the antecubital fossa, which had been exposed. Cotton soaked in rubbing alcohol was used to sterilize the area. The vein was then punctured with a 2 ml sterile disposable plastic syringe and a 24-gauge needle, and 2ml of blood was drawn. After the needle was removed, the tourniquet was released and cotton soaked in rubbing alcohol was applied to the puncture site. Blood was drawn and placed in a tube containing ethylenediaminetetraacetic acid.

2.4. Estimation of HbA1c in serum

Using the glucose oxidase method,[17] the levels were determined. Approximately 5 mins were spent centrifuging the sample at 3000rpm. One milliliter of glucose reagent was added to 10 l of test sample and standard glucose. Both were incubated for 10 mins at 37° Celsius. Using a semiautomatic analyzer, the absorbance values were measured and expressed as mg/dl.

2.5. Saliva collection

Saliva collections could be done by individuals with limited training and no special equipment is needed for collection. Diagnosis of disease based on the salivary analysis is potentially valuable for children and older adults, since the collection of fluid is associated with fewer compliance problems when compared with a collection of blood. Before having their saliva collected, patients were given strict instructions to abstain from eating and drinking for a period of 2 hrs. The samples were collected 2 hrs after the subject had consumed their breakfast. The technique known as “spitting” was used to collect the saliva that had not been stimulated.[18] The collection of saliva samples took place in the morning, specifically between the hours of 9 and 11 a.m. During this time samples were collected, the patient was instructed to remain in the dental chair with her head inclined forward and not to speak, swallow. The patients was instructed to spit their saliva into a clean graduated container once every minute for approximately 10 mins. Approximately 2 mL of saliva was collected each time. After centrifuging the saliva samples for 20 mins at three thousand revolutions per minute (rpm), the clear supernatants were analyzed to determine the levels of glucose, amylase, and total protein.

2.6. Estimation of salivary glucose

The glucose oxidase method was used with the help of a semi-automated analyzer[19] to determine the levels of glucose in the saliva. Combining the sample (100 l) and the reagent in a ratio of 1:3, the mixture was then heated to 37° Celsius for 5 mins. Compared to the absorbance values of the reagent blank, the values of the standard and sample were determined. In order to get an accurate reading of the glucose levels in the saliva, the standard was diluted by a factor of ten.[14]

2.7. Estimation of salivary amylase and total protein

The direct substrate kinetic enzymatic method[20] was used to calculate salivary amylase levels. The mean change in absorbance per minute were calculated and expressed as units per liter.[15,17] The endpoint method was used to estimate total protein using pyrogallol red dye.[18,19] The values were given in milligrams per deciliter.

Serum HbA1c levels and saliva samples were collected at baseline from 125 chronic periodontitis patients with type 2 diabetes in Group I. In addition, after 6 weeks of non-surgical periodontal therapy, HbA1c and saliva samples were collected in Group-I for CRP, glucose, amylase, and total protein analysis.

The same procedure was carried out on group II patients as well.

After six weeks of non-surgical periodontal therapy in both groups, all patients were objectively evaluated for HbA1c, CRP, glucose, amylase, and total protein.

2.8. Estimation of CRP in saliva

This is a sandwich ELISA that can be used indirectly.[21] When the pre-coated capture anti-CRP antibody on the plate binds CRP in standards and samples, a “sandwich” is formed. This “sandwich” is then bound by the anti-CRP detection antibody, which is linked to horseradish peroxidase. After each round of incubation, the unbound components are removed by washing. The reaction of the horseradish peroxidase (HRP) enzyme to the substrate tetramethylbenzidine is then used to measure the levels of bound anti-CRP Antibody Enzyme Conjugate (TMB). The color blue is produced as a result of this reaction. The reaction is halted by adding an acidic solution, which causes the formation of a yellow color. The optical density is measured with a conventional plate reader at 450nm. The amount of CRP that is present in a sample directly affects how well CRP Antibody Enzyme Conjugates are detected in that sample.

To estimate salivary CRP, the Salimetrics® Salivary CRP ELISA Kit (Generation II) was utilized. This kit is an enzyme-linked immunoassay that was developed and validated specifically for the quantitative measurement of human CRP in oral fluid.

2.8.1. Statistical analysis

For data entry, database administration, and statistical analysis, the Statistical Program for the Social Sciences (SPSS 24.0 version, Delaware, Chicago) software package was used. The student’s t-test was used to compare serum and salivary biomarkers in chronic generalized periodontitis subjects without diabetes to chronic generalized periodontitis patients with type II diabetes. The results were presented as means standard deviation, with a $p < 0.05$ was considered significant. Comparison of blood glucose levels, salivary glucose, amylase, and total protein was done by paired *t*-test. Intergroup correlations were calculated using Karl Pearson’s correlation coefficient.

3. Results

The age of patients included in the study ranges from 35 to 70 years.

As shown in Table 1; In Test Group, the assessed biomarkers measured in chronic generalized periodontitis patients with diabetes before and after non-surgical periodontal therapy showed a reduction in their levels, though it is not statistically significant with serum HbA1c, amylase, total protein, and glucose. The mean p-value for CRP was 0.028 ($p < 0.05$), a significant reduction probably due to reduced inflammation in the periodontal tissues post-therapy. Similarly, for control group, the comparisons between pre-operatively and post-operatively values, the mean p-value was 0.047 for CRP exhibited a significant reduction in inflammatory marker in chronic generalized periodontitis patients before and after treatment reduction in inflammation in the periodontal tissues after therapy. The rest of the levels of the assessed markers comprising serum $HbA1_c$, salivary amylase, total protein, and glucose showed a quantitative reduction, but not significantly. The study, however, showed that there was no marked difference in the assessed levels both pre- and post-operatively.

At baseline level (pre-operatively), the difference in levels when compared concerning the biomarkers between test and control group,

Table 1
Comparative mean values of parameters between genders at Baseline and Post OP in Test Group and Control group.

Parameters	Test group (Chronic periodontitis and Type-2 Diabetes) mean values					Control group (Chronic periodontitis and non-Diabetic) mean values				
	Male (n = 64)		Female (n = 61)		p value	Male (n = 83)		Female (n = 42)		p value
	Baseline	Post-Op	Baseline	Post-Op		Baseline	Post-Op	Baseline	Post-Op	
HbA1C	8.2812	8.2422	8.2195	8.1984	0.063	4.9590	4.9590	4.9714	4.9714	0.052
α Amylase	2.43E5	2.11E5	2.12E5	1.86E5	0.058	9.86E4	9.57E4	8.85E4	8.63E4	0.055
Total Protein	0.5003	0.5074	0.5452	0.4700	0.054	0.1193	0.1087	0.1045	0.1024	0.062
Salivary Glucose	2.5558	2.2100	2.6643	2.4105	0.057	0.5210	0.4916	0.4425	0.4174	0.063
C Reactive Protein	1.7925	1.5036	1.5066	1.2457	0.047	1.4816	1.4270	1.4993	1.4029	0.028

there was a highly significant difference with serum HbA_{1c} ($P < 0.000$), amylase ($P < 0.000$), total protein ($P < 0.000$), glucose ($P < 0.000$) except CRP ($P < 0.085$) which was not significant. The assessed levels have a varying range of measuring units, so they showed statistical differences when compared except hs-CRP. Similarly, post-operatively, i.e., after periodontal therapy, the difference in levels were compared between test and control group, there was a highly significant difference between serum HbA_{1c} ($P < 0.000$), amylase ($P < 0.000$), total protein ($P < 0.000$), glucose ($P < 0.000$) except CRP ($P < 0.638$) again. This study revealed that both post-therapy test and control group with and without diabetes demonstrated significant differences when compared to before non-surgical therapy, indicating that the activity of salivary biomarkers differed between diabetics and non-diabetic patients, despite the fact that all patients had periodontitis.

In test group, total of 64 males and 61 females were included. At a baseline level, there is no significant correlation exists between genders. Thus, it shows that gender does not affect HbA1c, amylase, CRP, glucose, and total protein values though minor changes were observed here. HbA_{1c} mean was more in males, 8.2812 compared to 8.2295 in females. Alpha-amylase more in men 2.43E5 compared to females 2.12E5. CRP was more in males with a mean of 1.7925 to females 1.5066. Total protein was observed more in females with a mean of 0.5452 to mean 0.5003. Glucose was more in females with a mean of 2.6643 to male 2.5558. Similarly, in this group, there was no significant correlation between genders on any of the assessed levels after therapy, with chronic generalized periodontitis with diabetes. HbA1c, amylase, CRP, glucose, and total protein showed negligible level differences. HbA_{1c} mean was more in males, 8.2422 compared to 8.1984 in females. Amylase was more in men with a mean of 2.11E5 compared to females with a mean of 1.86E5. Highly sensitive CRP more in male with mean 1.5036 compared to female mean 1.2457. Total protein was observed more in females with a mean 0.5074 to male mean 0.4700. Glucose was more in females with a mean of 2.4105 to male mean 2.2100.

In control group, total of 83 males and 42 females were included. There was no significant correlation with gender variation at a baseline level or on any of the assessed levels before treatment as observed. However, the mean readings of each assessed biomarkers showed minute changes among the two genders; in this Group II, the HbA_{1c} showed more in females with a mean of 4.9714 compared to 4.9590 in males. Alpha-amylase was observed more in males with a mean of 9.86E4 than females with an 8.85E4. Hs-CRP more in a female with a mean 1.4993 compared to 1.4816 in males. Total protein mean was observed more in males with a mean of 0.1193 to female mean 0.1045 and glucose more in men with a mean 0.5210 with 0.4424 in women. Similarly, the gender assessment after therapy in group II, post-therapy, also showed no significant changes except for some minor numerical differences in estimated units. HbA_{1c} was observed to be more in a female with a mean 4.9714 with male 4.9590. Amylase more in males, mean 9.57E4 compared to female mean 8.63E4. Hs-CRP was more in males with a mean of 1.4270 with female mean 1.4029. Total protein more in males; mean 0.1087 with female mean 0.1024. Glucose was more in males compared to females with a mean of 0.4916 to 0.4174.

4. Discussion

The primary goal of the study was to compare salivary CRP, amylase, total protein, and glucose levels in type 2 diabetic and non-diabetic patients with chronic periodontitis before and after non-surgical periodontal therapy. This study confirms the null hypothesis that there is no significant difference in salivary amylase, total protein, or glucose levels between test and control groups. However, there is a significant difference between the two groups at the baseline level, i.e., pre-operatively and 6 weeks post-operatively, i.e., there was a significant reduction in CRP levels at 6 weeks post-therapy in periodontitis group ($p < 0.05$).

Post-operative values were obtained after 6 weeks since this has been reported to be the minimal time required for the healing of periodontal tissues following non-surgical periodontal therapy; hence, any additional systemic advantages can only be anticipated after this time frame. Participants eligible for this study had at least ten natural teeth; nevertheless, diverse criteria have been identified in the literature for evaluating the severity of periodontal disease, and a consensus cannot be reached. Thus, we examined patients with a minimum of 10 teeth each arch.

Diabetes mellitus is a chronic disease that is characterized by a lack of insulin, cellular resistance to the action of insulin, or both. As a result, hyperglycemia and other metabolic disturbances are caused by diabetes mellitus. It is linked to severe complications of the eyes, kidneys, heart, and blood vessels, as well as other organ systems, all of which can reduce a patient's quality of life and potentially shorten their lifespan.[22] In this study HbA1c equal to or more than 7.0% was taken because evidence has shown that glycated haemoglobin levels of 7%–7.7% have yielded, the best results when treating patients with type 2 diabetes.

Periodontal disease is a chronic disease that consists of a group of inflammatory conditions that affect and disintegrate the dentition's supporting structures. The role of dental plaque biofilms in the etiology of periodontal diseases has been investigated. However, if not treated, the paradoxical impact of the susceptible host's inflammatory response to microbial challenges leads to the destruction of periodontal tissues and subsequent tooth loss. Diabetes is linked to periodontitis due to changes in basement membrane permeability, improper neutrophil chemotaxis, collagen synthesis breakdown, genetic predisposition, and increased susceptibility to periodontal pathogens.[23–25] Uncontrolled DM is more likely to result into tooth loss than for reduced number of teeth to precede the onset of DM.[26] A minimum of 20 teeth is required for the total periodontal pocket surface area to exert systemic effects from periodontitis. Saliva, in addition to ions and water, contains a complex mixture of thousands of proteins derived from various sources. Acinar cells synthesize and secrete most high-abundance proteins such as amylase, cystatins, and gustin, while duct cells of salivary glands secrete kallikreins and growth factors. However, hundreds of different types of proteins are found in saliva, and they are present in blood plasma too, implying that plasma proteins eventually make their way into saliva.[27]

The salivary biomarkers studied in this study serve as markers for a variety of disorders in oral conditions. In studies,[28] biomarkers such as glucose showed a reduction after non-surgical therapy. The total protein content of saliva increases in diabetic patients, oral cancer patients, and patients with history of dental caries.[29] C reactive protein, an

inflammatory marker, decreased in saliva after periodontal therapy, suggesting that it could be used as an inflammatory marker. Salivary amylase and total protein content are elevated in leukaemia patients' saliva and can thus be used as a markers too.[30]

However, despite the fact that both groups of patients with periodontitis, had no significant difference after 6 weeks. Among the assessed parameters, CRP levels decreased after therapy in group with periodontitis patients, which supports other studies. Vidal F et al., Veena A Patil and Manthan H Desai, Correa FOB et al.[31–33]

The other parameters like amylase total protein and CRP showed no difference after non-surgical periodontal therapy in the periodontitis group pre and post therapy, indicating that there were no significant changes in their levels following therapy. Among the assessed parameters, CRP levels were the only ones to change which supports similar findings in other studies.[34,35]

Because the assessed levels have a varying range of measuring units, they showed statistical differences when compared diabetic to the non-diabetic group, with the exception of CRP, which did not show any statistically significant difference. The current study found that when before therapy baseline levels in both groups, i.e., with and without diabetes, when compared, showed significant differences, indicating the activity of the biomarkers The levels of these markers differed between the chronic periodontitis groups that did not have diabetes and those that did; however, there was no significant difference in their levels after treatment. When compared to non-diabetics, people with diabetes had higher mean salivary glucose levels. Higher salivary glucose levels promote microorganism proliferation and colonization on teeth and oral mucous membranes. Significant increases in salivary amylase levels have also been observed in diabetics,[36–38] which confirms the results of this study. Several studies have supported amylase as a potential factor in streptococcal adhesion to teeth and plaque formation, its role in microorganism adhesion remains still a mystery. Studies have revealed that diabetics have higher salivary total protein levels than non-diabetics, which supports the findings this study.[39,40]

This study finds both post-therapy Groups I and II with and without diabetes showed a highly significant difference compared to each other, implying that the markers' activity and biochemical activity levels differed in the chronic periodontitis group without diabetes compared to with diabetes.

The deterioration of glycemic control associated with severe periodontitis is due to ongoing systemic challenges with periodontopathic bacteria and their products. It increases tissue insulin resistance, preventing glucose from entering target cells and resulting in elevated blood glucose levels.[41] Furthermore, salivary amylase levels had increased significantly.

It was also discovered that the gender difference had no effect on HbA1c, amylase, CRP, glucose, and total protein levels. Furthermore, there is no significant mean difference in salivary total protein, amylase, and glucose levels between males and females in all periodontitis groups, there were minor quantitative differences pre and post periodontitis but no significant differences in salivary glucose levels, despite the fact that males had higher salivary glucose levels than females.[42–45]

The current study's strength is the use of salivary biomarkers to estimate systemic inflammation caused by chronic periodontitis and its relationship to diabetes. Saliva collection is a non-invasive method of collecting samples, and salivary glucose levels correlated well with HbA1C. Also, type 2 diabetic subjects benefit decrease in their glucose levels as a result of nonsurgical periodontal therapy. This lends credence to the two-way relationship between periodontal disease and diabetes mellitus, as evidenced by the improvement in metabolic control brought about by the resolution of periodontal inflammation and infection.

4.1. Limitations

However, the current study has some limitations, for example small sample size. It is more challenging to collect, gingival crevicular fluid (GCF) was not used as a sample which could have been a better for accurate results. The use of saliva as a diagnostic tool for detection of biomarkers is prone to contamination and concentration variability of proteins and enzymes. Gingival crevicular fluid (GCF) could be a better sample for detecting inflammatory biomarkers since CRP levels in GCF and serum increase proportionally with the severity of periodontal diseases, and studies have demonstrated increased serum CRP levels as a result of a moderate inflammatory stimulus after periodontal therapy. Future studies with a larger sample size and using GCF as a sample could be used to address the limitations of the present study.

4.2. Clinical application

The utilisation of saliva for clinical and translational applications has risen to the fore. For early identification, disease progression, and therapy monitoring, the salivary proteome and salivary transcriptome are the most significant new tools for periodontal disorders. This study demonstrates that saliva has considerable therapeutic application potential as a biomarker. New technologies, such as lab-on-a-chip and microfluidic devices, offer the ability to handle complicated oral fluids, such as saliva and GCF, and to determine a patient's periodontal disease-risk profile, disease activity, and therapy response.[46–48] This biomarker strategy should expedite clinical decision-making and monitoring of episodic disease development in chronic infectious diseases like periodontitis. Moreover, saliva is easy to manage because it does not clot.[49,50] Salivary biomarkers if researched and promoted well could becomes more prevalent. With reduced costs they could become as effective and popular as urine/blood investigations. Due to unique sensitivity in its techniques, the cost of the saliavary assessment is presently high.

5. Conclusion

The purpose of the current study was to determine the potential of saliva as a biomarker, as well as the relationship between chronic generalized periodontitis with type 2 diabetes and patients with chronic generalized periodontitis without diabetes, as well as the selected salivary biomarkers HbA1c, Amylase, total protein, glucose, and CRP. The purpose of this study was to evaluate the association between these biomarkers and their response to non-surgical periodontal care, as well as their activity in both sexes. The use of saliva as a non-invasive glucose monitoring technique in Type 2 diabetes was also investigated before and after non-surgical periodontal therapy. CRP marker activity also showed a substantial differences. When the other parameters were compared before and after therapy, there were differences, but not statistically significant, indicating that CRP was a potent inflammatory marker with reduced decrease activity after therapy in both diabetic and non-diabetic individuals with chronic generalized periodontitis. Several researchers claim that good anti-infective periodontal therapy can reduce CRP levels in systemically healthy subjects, however this is not a consistent conclusion.

Several indicators, including blood HbA1c and salivary glucose, amylase, total protein, and CRP, were found to be elevated in diabetics with chronic periodontitis compared to non-diabetic patients. In both the test and control groups, there were no significant gender differences in parameters. The correlation between salivary glucose and HbA1C was strong. Hence, saliva has the potential to be utilized as a non-invasive glucose monitoring technique in Type 2 diabetes.

Funding

No Funding received.

Ethical approval

All of the procedures that were carried out in research projects that

included human participants were carried out in accordance with the ethical standards of the relevant institutional and/or national research committees, as well as the 1964 Helsinki declaration and any subsequent amendments or other ethical standards that were comparable.

Informed consent

It was obtained from all individual participants included in the study.

Declarartion of competing interest

The authors warrant that they do not have any competing interests to declare.

References

1 Haslett C, Chilvers ER, Chilvers ER, Hunter JA, Boon NA. *Davidson's Principles and Practice of Medicine*. eighteenth ed. Great Britain: Churchill Livingstone; 2000:472.
2 Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047–1053.
3 Abikshyeet P, Ramesh V, Oza N. Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes Metab Syndr Obes*. 2012;5:149–154.
4 Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2003;26(suppl 1). S5-20.
5 Manfredi M, McCullough MJ, Vescovi P, Al-Kaarawi ZM, Porter SR. Update on diabetes mellitus and related oral diseases. *Oral Dis*. 2004;10:187–200.
6 Vernillo AT. Dental considerations for the treatment of patients with diabetes mellitus. *J Am Dent Assoc*. 2003;134, 24S-33.
7 Kaufman E, Lamster IB. The diagnostic applications of saliva – a review. *Crit Rev Oral Biol Med*. 2002;13:197–212.
8 Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. *J Oral Sci*. 2010;52:359–368.
9 Belazi MA, Galli-Tsinopoulou A, Drakoulakos D, Fleva A, Papanayiotou PH. Salivary alterations in insulin-dependent diabetes mellitus. *Int J Paediatr Dent*. 1998;8:29–33.
10 Vasconcelos AC, Soares MS, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in Type 2 diabetic patients. *J Oral Sci*. 2010;52:293–298.
11 Ephraim RKD, Anto EO, Acheampong E, et al. Fasting salivary glucose levels is not a better measure for identifying diabetes mellitus than serum or capillary blood glucose levels: comparison in a Ghanaian population. *Heliyon*. 2019;5(3), e01286.
12 Kumar A, Kumar T, Bhargava M, Raj R, Vaibhav V, Kishore J. Salivary and serum glucose levels in diabetes mellitus patients versus control - a randomised control trial. *J Med Life*. 2020;13(2):235–240.
13 Little RR, Goldstein DE. Endocrine (standardization of glycohemoglobin measurement). *Anal Chem*. 1995;67, 393R-7.
14 Arias-Bujanda N, Regueira-Iglesias A, Balsa-Castro C, Nibali L, Donos N, Tomás I. Accuracy of single molecular biomarkers in saliva for the diagnosis of periodontitis: a systematic review and meta-analysis. *J Clin Periodontol*. 2020;47(1):2–18.
15 Khorsand A, Paknejad M, Yaghobee S, et al. Periodontal parameters following orthodontic treatment in patients with aggressive periodontitis: a before-after clinical study. *Dent Res J*. 2013;10(6):744–751.
16 Yuen VG, McNeill JH. Comparison of the glucose oxidase method for glucose determination by manual assay and automated analyzer. *J Pharmacol Toxicol Methods*. 2000;44(3):543–546.
17 Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci*. 1993;694:72–77.
18 Kumari S, Bahinipati J, Pradhan T, Sahoo DP. Comparison of test performance of biochemical parameters in semiautomatic method and fully automatic analyzer method. *J Fam Med Prim Care*. 2020;9(8):3994–4000.
19 Seibert E, Tracy TS. Fundamentals of enzyme kinetics. *Methods Mol Biol*. 2014;1113:9–22.
20 Gonzalez RM, Seurynck-Servoss SL, Crowley SA, et al. Development and validation of sandwich ELISA microarrays with minimal assay interference. *J Proteome Res*. 2008;7(6):2406–2414.
21 Franco OH, Steyerberg EW, Hu FB, Mackenbach J, Nusselder W. Associations of diabetes mellitus with total life expectancy and life expectancy with and without cardiovascular disease. *Arch Intern Med*. 2007;167(11):1145–1151.
22 Belstrøm D, Constancias F, Drautz-Moses DI, et al. Periodontitis associates with species-specific gene expression of the oral microbiota. *NPJ Biofilms Microbiomes*. 2021;7(1):76.
23 Mealey BL, Oates TW. American Academy of Periodontology. Diabetes mellitus and periodontal diseases. *J Periodontol*. 2006;77(8):1289–1303.
24 Soskolne WA, Klinger A. The relationship between periodontal diseases and diabetes: an overview. *Ann Periodontol*. 2001;6(1):91–98.
25 Llambés F, Arias-Herrera S, Caffesse R. Relationship between diabetes and periodontal infection. *World J Diabetes*. 2015;6(7):927–935.
26 Weijdijk LPM, Ziukaite L, Van der Weijden GAF, Bakker EWP, Slot DE. The risk of tooth loss in patients with diabetes: a systematic review and meta-analysis. *Int J Dent Hyg*. 2022;20(1):145–166.
27 Rathnayake N, Akerman S, Klinge B, et al. Salivary biomarkers of oral health: a cross-sectional study. *J Clin Periodontol*. 2013;40(2):140–147.
28 Melguizo-Rodríguez L, Costela-Ruiz VJ, Manzano-Moreno FJ, Ruiz C, Illescas-Montes R. Salivary biomarkers and their application in the diagnosis and monitoring of the most Common oral pathologies. *Int J Mol Sci*. 2020;21(14):5173.
29 Alamoudi A, Alamoudi R, Gazzaz Y, Alqahtani AM. Role of salivary biomarkers in diagnosis and detection of dental caries: a systematic review. *Diagnostics*. 2022;12(12):3080.
30 Negrini TC, Carlos IZ, Duque C, Caiaffa KS, Arthur RA. Interplay among the oral microbiome, oral cavity conditions, the host immune response, diabetes mellitus, and its associated-risk factors-an overview. *Front Oral Health*. 2021;2, 697428.
31 Yang Y, Liu S, Wang Y, et al. Changes of saliva microbiota in the onset and after the treatment of diabetes in patients with periodontitis. *Aging (Albany NY)*. 2020;12(13):13090–13114.
32 Matczuk J, Żendzian-Piotrowska M, Maciejczyk M, Kurek K. Salivary lipids: a review. *Adv Clin Exp Med*. 2017;26(6):1021–1029.
33 Vidal F, Cordovil I, Figueredo CM, Fischer RG. Non-surgical periodontal treatment reduces cardiovascular risk in refractory hypertensive patients: a pilot study. *J Clin Periodontol*. 2013;40(7):681–687.
34 Patil VA, Desai MH. Effect of periodontal therapy on serum C-reactive protein levels in patients with gingivitis and chronic periodontitis: a clinicobiochemical study. *J Contemp Dent Pract*. 2013;14(2):233–237.
35 Correa FO, Gonçalves D, Figueredo CM, Bastos AS, Gustafsson A, Orrico SR. Effect of periodontal treatment on metabolic control, systemic inflammation and cytokines in patients with type 2 diabetes. *J Clin Periodontol*. 2010;37(1):53–58.
36 Lasisi TJ, Fasanmade AA. Comparative analysis of salivary glucose and electrolytes in diabetic individuals with periodontitis. *Ann Ib Postgrad Med*. 2012;10(1):25–30.
37 Ashok L, Sujatha GP, Hema G. Estimation of salivary amylase and total proteins in leukemia patients and its correlation with clinical feature and radiographic finding. *Indian J Dent Res*. 2010;21(4):486–490.
38 Tabatabaei F, Mahjoub S, Alijanpour M, et al. Evaluation of the relationship between salivary lipids, proteins and total antioxidant capacity with gingival health status in type-1 diabetic children. *J Dent*. 2021;22(2):82–89.
39 Pérez-Ros P, Navarro-Flores E, Julián-Rochina I, Martínez-Arnau FM, Cauli O. Changes in salivary amylase and glucose in diabetes: a scoping review. *Diagnostics*. 2021;11(3):453.
40 Malathi L, Masthan KM, Balachander N, Babu NA, Rajesh E. Estimation of salivary amylase in diabetic patients and saliva as a diagnostic tool in early diabetic patients. *J Clin Diagn Res*. 2013;7(11):2634–2636.
41 Panchbhai AS. Correlation of salivary glucose level with blood glucose level in diabetes mellitus. *J Oral Maxillofac Res*. 2012;3(3):e3.
42 Steigmann L, Maekawa S, Kauffmann F, et al. Changes in salivary biomarkers associated with periodontitis and diabetic neuropathy in individuals with type 1 diabetes. *Sci Rep*. 2022;12(1), 11284.
43 Lundmark A, Hu YOO, Huss M, Johannsen G, Andersson AF, Yucel-Lindberg T. Identification of salivary microbiota and its association with host inflammatory mediators in periodontitis. *Front Cell Infect Microbiol*. 2019;9:216.
44 Qin H, Li G, Xu X, et al. The role of oral microbiome in periodontitis under diabetes mellitus. *J Oral Microbiol*. 2022;14(1), 2078031.
45 Silva DNA, Casarin M, Monajemzadeh S, Bezerra BB, Lux R, Pirih FQ. The microbiome in periodontitis and diabetes. *Front Oral Health*. 2022;3, 859209.
46 Simpson TC, Clarkson JE, Worthington HV, et al. Treatment of periodontitis for glycaemic control in people with diabetes mellitus. *Cochrane Database Syst Rev*. 2022;4(4), CD004714.
47 Brand HS, Veerman EC. Saliva and wound healing. *Chin J Dent Res*. 2013;16(1):7–12.
48 Špiljak B, Šimunović L, Lapić I, Rogić D, Špalj S, Vuletić L. Influence of saliva on the results of global laboratory coagulation tests. *Aust Dent J*. 2020;65(3):205–209.
49 Deutsch O, Palmon A, Aframian DJ. [Screening saliva]. *Refuat Ha-Peh Veha-Shinayim*. 2013;30(2):16–23, 78. Hebrew.
50 Patil PB, Patil BR. Saliva: a diagnostic biomarker of periodontal diseases. *J Indian Soc Periodontol*. 2011;15(4):s310–s317.