



Evaluation of the Effect of Routine Periodontal Treatment (SRP and Oral Hygiene Instruction) on Salivary Characteristics and Blood Sugar Control in Diabetic Patients: A Clinical Trial

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Abstract

Background: Diabetes mellitus is quite prevalent and causes many complications in different parts of the body. It also affects the quality of life by causing many complications in the oral cavity. This study examined a simple intervention to improve oral and periodontal tissue health and investigate its effect on the patients salivary characteristics and blood sugar control.

Methods: This double-blind clinical trial was performed on 40 patients with type 2 diabetes. Fasting blood sugar (FBS), glycated hemoglobin (HbA1C), salivary glucose, amylase, total protein, viscosity, and pH were evaluated before and after scaling and oral hygiene instructions. The obtained data were entered into SPSS 17 statistical software and analyzed using the *t*-test, Mann-Whitney, and chi-square tests.

Results: After the intervention, the FBS results in both groups (*P* value=0.015 in the case group and *P* value=0.017 in the control group) and HbA1C in the case group (*P* value=0.006) decreased significantly. Also, the difference in salivary viscosity, pH, and glucose between the case group and the control group was significant (*P* value=0.005, 0.008, and 0.021, respectively).

Conclusion: Based on the results of the present study, scaling, and oral hygiene instructions reduce FBS and HbA1C, salivary glucose and amylase and oral conditions, leading to good blood sugar control.

Keywords: Diabetes mellitus, Scaling, Root planing, Salivary characteristics, Blood glucose

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Introduction

Diabetes mellitus is a group of metabolic diseases, including high blood sugar resulting from a defect in insulin secretion, insulin function, or both. Two types of diabetes can be identified: type 1 and type 2. The WHO currently recognizes type 2 diabetes as the sixth cause of death worldwide (1-4).

Effective diagnosis of diabetes is a crucial strategy to reduce the incidence and complications of the disease. Diabetes affects the quality of life by causing multiple complications in the oral cavity (5). Evidence has shown a bi-directional link between diabetes mellitus and periodontitis (6,7). According to previous research, the rate of periodontal destruction is higher in diabetic patients over 30 years of age. The severity and rate of progression of periodontal disease and gingivitis are also higher in

these patients (8). According to studies, scaling and root planing (SRP) improve periodontal status and decrease fasting blood sugar and glycated hemoglobin (HbA1C) (9). diabetes affects the function and composition of saliva, including its pH. It ultimately changes the oral cavity's qualitative and quantitative characteristics, increasing caries and dental plaque (10).

Glucose, amylase, and total protein in saliva are closely related to the quality of the oral environment in patients with type 2 diabetes. Studies have shown that saliva viscosity, total protein, amylase, and glucose are higher in diabetic patients than non-diabetics, and the salivary pH in people with diabetes is lower than in non-diabetics (11,12).

Changes in saliva components can effectively reduce oral symptoms and complications in diabetic patients.



According to available studies, saliva glucose levels reflect the blood glucose levels in diabetic patients (10,11).

Non-invasive glucose determination techniques have advantages over invasive methods. They are less dependent on patient cooperation, affordable, and suitable for screening a large population. Recently, a rapid chair-side test to evaluate saliva has been introduced. It evaluates saliva properties such as hydration, consistency, pH, stimulated and unstimulated salivary flow, and buffer capacity (9,11).

Diabetes is quite prevalent and causes many complications in different parts of the body. One of the complications of diabetes mellitus that has received less attention is its effects on oral tissues and the salivary glands. As one of the most important complications of diabetes is dry mouth and periodontal disease, this study aimed to perform a simple intervention in oral and periodontal tissue health and evaluate its effect on patients' saliva characteristics and blood sugar control.

A review of available resources shows that many previous studies were observational. Therefore, the present study was designed as a clinical trial that recorded the difference between some important parameters of saliva and blood sugar before and after the intervention. The present study was comprehensive, evaluating both the physical factors of saliva, including viscosity and pH, and the chemical factors of saliva, i.e., salivary total protein, glucose, and amylase, while many previous studies evaluated only a few of these parameters.

Methods

This double-blind clinical trial involved 40 patients with type 2 diabetes who referred to Yazd Diabetes Research Center.

Patients from Yazd Diabetes Research Center with type 2 diabetes, identified by fasting blood sugar (FBS) ≥ 126 mg/dL and HbA1C $\geq 6.5\%$ (13) in a blood test from the last three months, who had no other comorbidity, were treated only with oral hypoglycemic drugs, and had generalized chronic periodontitis (14) were included in the study.

Patients who are unwilling to cooperate and who needed insulin injections or any changes in their medication regimen during the study period were excluded.

After obtaining patients' informed consent, data were obtained using a checklist and form comprising their demographic information, including age and gender, medical information according to their health files at the diabetes research center, and blood glucose test results (FBS and HbA1C).

Non-stimulated whole saliva samples were taken under standard conditions to assess glucose, pH, saliva viscosity, total protein, and amylase.

The amount of 2 cc of cumulative non-stimulated whole saliva was collected by asking patients to spit

into a container to obtain a saliva sample. The patients were asked to refrain from eating, drinking, smoking, and brushing for 90 minutes before sampling to reduce the effect of daily changes in saliva composition. Before collecting the samples, people were asked to sit and relax in a chair, collect their saliva, and empty it into a special sterilized container. They were asked to repeat this every 60 seconds for 5 minutes (15).

Patients were divided into two groups based on their preference for intervention. The first group was trained to brush their teeth twice a day using the modified bass method to standardize health education among all participants. Also, scaling and root planing were performed using Cavitron, Dentsply, York, PA ultrasonic devices by the researcher in the dental unit of the Diabetes Research Center.

The second group, which was considered the control group, received oral hygiene instruction in a similar manner. The case and control groups were also matched in age and gender. After 3 months, the same factors were evaluated under the same conditions, and the results of FBS and HbA1C were re-evaluated and recorded.

A pen-type digital pH meter (AZ Taiwan) was used to evaluate the pH of saliva. The viscosity of the samples was measured based on the amount of saliva sample movement in a capillary tube calibrated in millimeters. A higher amount of saliva movement is indicative of lower viscosity. In order to measure the amount of saliva movement in millimeters per second, the capillary tube was placed in a saliva sample container for 10 seconds, and the amount of saliva rising was measured in millimeters. In order to reduce researcher error for each saliva sample, the test was done twice, and the average value was recorded.

The viscosity of the control liquids, including glycerin and water at 25 °C, was determined by a capillary tube (830 mm/2 s and 1 mm/2 s, respectively).

Then, the saliva samples were immediately sent to the laboratory for glucose, total protein, and amylase evaluation. Saliva samples were centrifuged at 4400 rpm for 15 minutes to isolate impurities. The isolated samples were then pipetted into plastic tubes, paraffinized, and then frozen at -70 °C in a UK-made U410 freezer to inactivate the glycolysis cycle of glucose uptake by bacteria for further testing. Saliva samples were analyzed using a glucose oxidase /peroxidase kit (Biosystems S.A. Costa Brava30 CO., Barcelona, Spain) with a detection range of at least 0.23 mg/dL in Bouali laboratory in Yazd. For this purpose, standard glucose concentrations of 0.312, 0.625, 1.25, 2.5, 5, and 10 were prepared. Then, 100 λ was poured into the test tubes separately, and 1000 λ reagent was added. Then, the incubator was maintained for 30 minutes at 37 °C. The absorption of concentrations was measured using a spectrophotometer. The standard curve was drawn in Excel according to the device's concentration and absorption

reading. Then, 100 λ from each saliva sample was poured into separate test tubes, and 1000 λ reagent was added. The samples were again placed in the incubator at 37 °C for 30 minutes. The adsorption rate of each saliva sample was reread by spectroscopy, and the glucose concentration of the saliva samples was obtained (16).

Salivary total protein evaluation was performed using pyrogallol red. Pyrogallol red forms a blue complex with a protein in an acidic environment containing molybdate ions. It is read at a wavelength of 600 nm, and its color intensity is proportional to the amount of sample protein. After obtaining the absorption coefficient of the saliva sample and based on the absorption coefficient of the calibrated solution, the amount of protein was calculated using the formula: Protein (mg/dL) = $A_{\text{sample}} / A_{\text{calibrator}} \times 50$; also, the enzymatic kinetic method was used to evaluate salivary amylase, which calculates the change in average absorption per minute (8).

This study was approved by the Research Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, with ethics code No. IR.SSU.REC.1399.144, and registered in the Iran Clinical Trial Center with the code IRCT20200B23048485N1.

The data were analyzed using SPSS 17 statistical software and evaluated separately for each variable. The results were compared between the two groups. For statistical analysis, the *t*-test was used to compare the results of saliva viscosity and pH, and the Mann-Whitney test was used to compare the results of blood sugar, salivary amylase, and total protein. Also, chi-square was used to compare age and gender. The α coefficient was considered 0.05 in all calculations.

Results

In this double-masked clinical trial, 40 diabetic patients were studied. Twenty were placed in the first group (case group) and received SRP and oral hygiene instruction. The other 20 were placed in the second group (control group) and only received oral hygiene instruction.

Table 1 provides basic information about the patients in each group. There was no significant difference between the two groups regarding age and gender.

The normality of data distribution was assessed using the Shapiro-Wilk method. Based on the obtained results, the viscosity and pH of saliva were not normally distributed. Therefore, non-parametric tests were used

to evaluate them, but other data were analyzed using parametric tests.

The blood glucose levels of patients before and three months after the study were compared using Mann-Whitney analysis, which showed that FBS values were significantly reduced three months after intervention in both groups (in group 1, $P=0.015$, and in group 2, $P=0.017$).

Also, HbA1C in the case group decreased significantly after scaling ($P=0.006$), and the mean difference of HbA1C in the case group was significantly higher than the control group ($P=0.047$) (Table 2).

In the case group, salivary pH after the intervention increased significantly to an average value of 0.49 ($P<0.0001$), but in the control group, this difference was insignificant. The rate of pH change in the case group was significantly higher than the control group ($P=0.008$) (Table 3).

The difference in saliva movement in the capillary tube to assess saliva viscosity decreased significantly after 3 months in the case group, while in the control group, this difference was not significant. Also, this difference in the case group was significantly greater than that in the control group ($P=0.005$) (Table 3).

Salivary glucose in the case group decreased significantly after the intervention ($P\text{-value}=0.02$). The mean difference in pH, viscosity, and glucose in the case group was significantly higher than that in the control group (Figure 1).

Discussion

Diabetes mellitus leads to quantitative and qualitative changes in saliva, eventually leading to changes in the oral cavity. Changes in salivary components can affect the symptoms and severity of oral manifestations. Some researchers believe saliva can be used as a diagnostic fluid to diagnose diabetes (10,17).

Diabetes mellitus and periodontal disease are interrelated. Diabetes destroys periodontium, which negatively affects blood sugar control in patients with type 2 diabetes (18). SRP accompanied by oral hygiene reduce periodontal infections and microbial agents and reduce blood sugar in patients by reducing inflammation (19).

The present study found a significant decrease in the mean FBS of the case and control groups after the intervention (Table 2). HbA1c was also evaluated for a longer evaluation of glycemic control, and HbA1C decreased significantly in the case group after 3 months.

In the study of Amini and Mohammadi (19) on diabetic patients after SRP, fasting blood glucose decreased from 184.20 ± 61.11 to 163.87 ± 29.08 mg/dL, and also HbA1C decreased from 8.78 to 7.29, which is statistically significant. The results of the present study are consistent with Amini and Mohammadi's findings (19). The present study's findings are comparable to a systematic review

Table 1. Biographic information of diabetic patients in the two groups

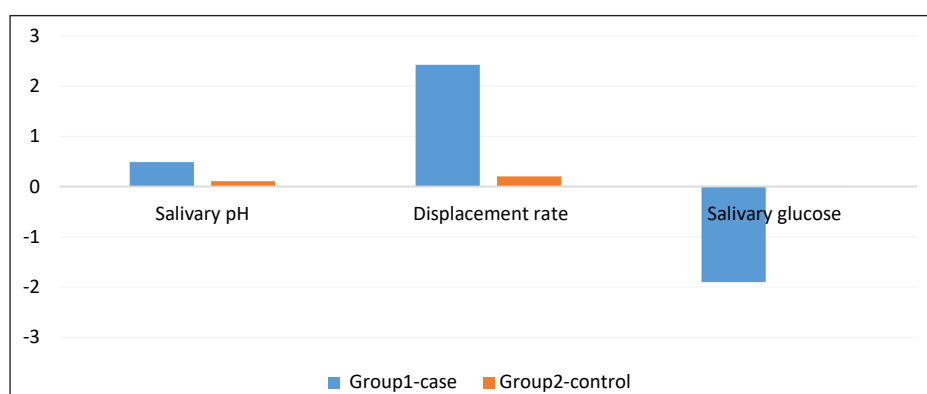
		Groups	
		Group 1(case)	Group 2 (control)
Number		20	20
Age (year)		49.75 ± 7.72	52.10 ± 8.96
Gender	Male	8 (40%)	11 (55%)
	Female	12 (60%)	9 (45%)

Table 2. Comparison of blood glucose results of diabetic patients before and after the intervention

Variable	Group	Before intervention	After intervention	P-value	Mean difference	P value
FBS (mg/dL)	Case	194.6±73.08	170.15±69.32	0.015	-24.45±38.79	0.088
	Control	167.55±36.58	156.45±43.34	0.017	-11.1±17.98	
HbA1C (%)	Case	7.84±1.60	7.41±1.28	0.006	-0.42±7.72	0.047
	Control	7.56±1.11	7.49±1.03	0.304	-0.06±0.34	

Table 3. Comparison of some salivary factors in type 2 diabetic patients before and after the intervention

Variable	Group	Before intervention	After intervention	P-value	Mean difference	P value
pH	Case	6.25±0.45	6.74±0.36	0	0.49±0.46	0.008
	Control	6.70±0.45	6.81±0.45	0.213	0.11±0.39	
Saliva movement (mm/10 s)	Case	9.42±2.97	11.85±2.49	0	2.42±2.29	0.005
	Control	11±3.12	11.20±3.28	0.711	0.20±2.37	
Glucose (mg/dL)	Case	6.90±10.44	4.99±8.10	0.021	-1.90±5.81	0.06
	Control	2.44±3.45	2.46±3.46	0.311	0.01±0.43	
Total protein (mg/dL)	Case	262.70±170.62	269.30±195.65	0.881	6.60±244.47	0.871
	Control	221.40±164.48	192.55±80.19	0.709	-28.85±1164.31	
Amylase (mg/dL)	Case	17432.10±27950.65	16158.90±26677.13	0.502	01273.5±34615.28	0.935
	Control	22509±29859.15	16547.85±21109.19	0.351	-5961.15±33192.37	

**Figure 1.** Comparison of the mean difference of some salivary factors of diabetic patients after the intervention

study (18) in which the patient's blood glucose was evaluated and compared before and after periodontal interventions. In one of the studies reviewed in this article, HbA1C decreased by 0.73 in diabetic patients with an average age of 59.62 years after scaling and health education (20). In another similar study, HbA1C decreased from 7.68 to 7.10 and FBS from 210.5 to 181.7 in the case group after scaling, but no difference was reported in the control group (21). Our research findings are consistent with these studies.

Based on previous studies, the salivary glucose levels in diabetic patients are higher than in non-diabetics due to the leakage of serum-derived glucose components into saliva through the gingival groove fluid. Also, diabetic patients have higher membrane permeability due to changes in microvascular processes in blood vessels caused by hyperglycemia. The sugar molecule is small and, therefore, can pass through a semi-permeable membrane,

causing glucose leakage into saliva (17).

In the present study, salivary glucose in the case group decreased after SRP, but the difference was not statistically significant. In various studies, the average fasting sugar in the saliva of diabetic patients has been evaluated and compared with non-diabetic patients. However, no study has evaluated the changes in saliva after periodontal treatment. The results of some studies (8,11,16,17) have shown that in diabetic patients, saliva sugar is higher than in normal people, so it can be concluded that with a decrease in blood sugar, saliva sugar decreases too. In the present study, both blood sugar and saliva sugar in patients decreased on average after the interventions, so it can be concluded that the results of the present study are consistent with other observational studies (8,11,16,17).

Mean salivary glucose assessments have shown different results in different studies, which can be related to the conditions and method of sample collection, the time

interval between sampling and sending the samples to the laboratory, and different laboratory methods, as well as changes in patient's diabetes control status, medications, and age of patients. In Mahdavi and colleagues' study (16), the method of saliva collection and the related tests were similar to those of the present study; the average saliva sugar in diabetic patients was 6.10, which was relatively similar to the present study.

Amylase is an enzyme involved in the hydrolysis of starch to the structural monosaccharide glucose. In diabetic patients, the level of the C-AMP receptor and amylase in the parotid gland, which is involved in the production of secretory protein in the salivary glands, is different (compared to non-diabetics), leading to changes in salivary amylase levels (17).

Conversely, increased permeability in the basement membrane is observed in diabetic patients. As a result, changes in membrane permeability and enzyme expression in the parotid gland lead to amylase leakage in the secreted saliva in these patients (2).

In the present study, the mean amylase levels in the case and control groups decreased, but the differences were not significant in the two groups.

Due to the same reasons mentioned in relation to saliva sugar, different studies have reported different results on salivary amylase. In addition, the type of saliva evaluated (stimulatory or non-stimulatory) affects the results of amylase concentration in patients. Salivary amylase is higher in diabetic patients than in control patients in all existing studies, which is consistent with the results of the present study. The mean salivary amylase in Tiongo (17) study was 930.827. In Kheirmand Parizi and colleagues' study (2), the amount of salivary amylase in patients with controlled diabetes and uncontrolled diabetes group was reported as 95793 and 161852, respectively. In Anjum and colleagues' study, diabetic patients had an average salivary α -amylase concentration of 12.06 ± 2.36 U/L, whereas it was 3.1 ± 0.88 U/L in the control group. Thus, the mean salivary α -amylase level was found to be much higher in diabetic patients than in the control group, and this difference was statistically significant ($P < 0.001$) (12).

Saliva comprises many proteins, including major glycoproteins such as mucin, proline-containing glycoproteins, and immunoglobulins, as well as minor proteins including agglutinin, lactoferrin, cystatin, and lysosomes (22). Different factors can affect salivary proteins. Increased activity of microorganisms can be associated with increased salivary protein. Also, increased basement membrane permeability in diabetic patients is one possible reason for the passage of protein from the exocrine glands to their secretions (9).

Total protein levels are affected by various factors such as saliva collection method, daily fluctuations in protein levels, and even centrifuge speed (10).

In the present study, no significant difference in the

mean protein levels was found in either of the studied groups. These findings are compatible with those of Ben-Aryeh et al (23), who reported that the mean salivary protein in diabetic patients was 223 ± 146 mg/dL.

Decreased salivary volume is common in diabetic patients, which can be explained by frequent urination and neurological and pathological changes in the salivary glands. Decreased salivary volume results in decreased pH. One of the complications of diabetes mellitus is changes in the salivary buffering system, which mainly contains bicarbonate and phosphate, which reduce the pH value of saliva (24).

In the present study, the mean pH value increased in both groups after SRP and oral health instruction. The rate of pH change in the case group was significantly higher than in the control group. These findings are relatively similar to the results reported by Owlia et al (24), in which the average salivary PH in diabetic patients was reported to be 6.11 for patients with a mean age of 51.21 years

Slight differences in results could be related to the different populations and the pH measurement method; in Owlia and colleagues' study (24), saliva pH was assessed using graded paper. In contrast, a digital pH meter was used in the present study, which has higher accuracy than graded paper. In this study, the average pH was lower in diabetic patients than healthy people. Since no similar intervention study was found to compare and validate the obtained results with previous studies, it can be concluded that by controlling diabetes and lowering blood sugar, the rate of salivary acidity decreases, which is consistent with the results of the present study.

As mentioned earlier, saliva viscosity is assessed by saliva movement in the capillary tube. Diabetes is associated with increased concentrations of saliva proteins and other salivary factors that can stimulate and inflame the mucosa and reduce saliva's cleansing properties, leading to numerous oral problems (25).

In the present study, the mean viscosity in the case group showed a significant decrease after 3 months, while in the control group, this difference was not significant. Various studies have shown that salivary viscosity is higher in diabetic patients than in normal individuals, which is consistent with the results of the present study. In Mohiti and colleagues' study (25), salivary viscosity in diabetic patients was significantly higher than in the control group, corresponding to the present study findings. One of the main reasons for the increase in the viscosity of non-stimulated saliva in diabetic patients can be explained by the increase in the concentration of proteins and other inflammatory factors, which leads to an increase in saliva concentration and viscosity.

The major limitation of the present study is that it was conducted during the COVID-19 pandemic, which made coordination between the dentist, the diabetes center, the laboratory, and patients more difficult.

Considering the importance of this subject, it is suggested that similar studies be done with the cooperation of more internal specialists/endocrinologists and medical centers and with larger sample sizes.

Conclusion

Based on the results of the present study, SRP can reduce fasting blood sugar and HbA1C, as well as salivary glucose and salivary amylase. Therefore, they help improve blood sugar control and oral conditions. In addition, careful training to improve oral hygiene can lead to positive changes in diabetes control and the patient's oral and salivary conditions. However, these instructions should be continuous and done with careful cooperation and planning to produce more accurate results.

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Authors' Contribution

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Project administration: Hakimeh Ahadian.

Resources: Hakimeh Ahadian, Arezoo Heidary.

Software: Hakimeh Ahadian, Arezoo Heidary.

Supervision: Hakimeh Ahadian.

Validation: Hakimeh Ahadian.

Visualization: Hakimeh Ahadian, Arezoo Heidary.

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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This study was approved by the Research Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, with the ethics code IR.SSU.REC.1399.144.

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