

Comparison of pH and Viscosity of Unstimulated Saliva in Type 2 Diabetic Patients and Control Group

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Abstract

Objectives: Diabetes mellitus is one of the most common chronic diseases that may cause irreversible complications. This disease can affect the salivary glands and oral health. Among physical and chemical alterations, changes in the pH and viscosity of saliva are particularly important. The aim of this study was to compare the pH and viscosity of unstimulated saliva in diabetic patients and non-diabetic controls.

Methods: In the present case-control study, three groups consisted of 36 controlled type 2 diabetic patients, 36 uncontrolled type 2 diabetic patients and 36 healthy controls were recruited and matched by age and sex. Their unstimulated cumulative saliva was collected for five minutes by the spitting method and the pH of samples was measured by a digital pH meter. The viscosity of saliva samples was assessed by comparing the sample displacement rate with that of control fluids at mm/10 seconds. Data were analyzed by SPSS version 20 via ANOVA and Bonferroni multiple comparisons test. P value less than 0.05 was considered statistically significant.

Results: A significant inverse correlation was found between the saliva pH and hyperglycemia ($P < 0.0001$). A significant relationship was noted between viscosity and severity of diabetes mellitus ($P < 0.0001$). The pH of saliva in uncontrolled diabetic patients was significantly lower than that of controlled type 2 diabetic patients and non-diabetic control group ($P < 0.05$). The viscosity of saliva in diabetic patients with well and poorly controlled hyperglycemia was more than that of the control group ($P < 0.009$ and $P < 0.0001$, respectively).

Conclusion: Diabetes mellitus causes a reduction in saliva pH and increases the viscosity of saliva, which can cause qualitative and quantitative changes in the saliva and oral health.

Key Words: Saliva; Viscosity; Hemoglobin A, Glycosylated; Diabetes Mellitus, Type 2

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Introduction

Diabetes mellitus is associated with vascular and metabolic changes. Increased blood glucose is associated with abnormal metabolism of glucose, lipid and protein that is due to partial or complete lack of insulin (1). The prevalence of diabetes mellitus among ethnic groups around the world is between 7.8% to 15.5% (2) and it is estimated that this rate will reach 439 million adults by 2030 in the world (3). The prevalence of diabetes mellitus was reported to be 9.3%

among 20-79-year-old Iranians by the International Diabetes Federation in 2010. Also, more than 1% of the urban populations over 20 years are diagnosed with type 2 diabetes mellitus in Iran every year (4). Yazd province with the prevalence of 16.3% has a high prevalence of diabetes mellitus (5). Diabetes mellitus has a significant relationship with coronary heart disease and causes organ damage affecting the kidneys, eyes and the nervous system in long-term. Glycosylated hemoglobin (HbA1c) is a standard measure that shows average blood

glucose over the past 2-3 months and it is used as the best measure for long-term control of diabetes mellitus (6). Diabetes mellitus directly and indirectly changes the quantity and quality of saliva which in turn can threaten oral health and dental tissues. Saliva is responsible for preservation of the health of oral soft tissues (7). The quantity and quality of saliva change in diabetic patients, which can result in dental caries, periodontal disease and oral mucosal lesions. Poor control of hyperglycemia is one reason for developing gingivitis, alveolar bone loss, salivary gland dysfunction and impaired sense of taste (8). Moreover, dry mouth following reduction in volume of saliva is common in most diabetic patients due to polyuria and neurological and pathological changes in the salivary glands (2).

The pH of the saliva is an important physical property of the saliva, responsible for its buffering capacity. Maintaining the acidity of the oral environment and the buffering action of the saliva are among the most important actions of saliva that protect the teeth. Viscosity is another important physical property of the saliva which also protects the oral cavity (9). Viscosity depends on several factors including protein content, presence of mucoproteins, inorganic compounds and the ambient temperature and these factors have a direct effect on the contents of saliva. Secretion of proteins into the saliva leads to change in viscosity and consequent change in properties of the saliva and may have irreparable effects on oral health (10).

Thus, it is important to evaluate the effect of these changes on the oral cavity. Maintaining oral health and systemic health is a basic requirement, because it has been proven that oral health has a direct impact on general health. As the prevalence of diabetes mellitus

has significantly increased in the recent years and considering its adverse effects on oral health, the aim of this study was to evaluate the pH and viscosity of the unstimulated saliva in diabetic patients in comparison with non-diabetic controls.

Methods

In this case-control study, 108 subjects (72 diabetics and 36 healthy controls) that were referred to Yazd Diabetes Research Center in the age range of 30-70 years were recruited and divided into three equal groups. The three groups included poorly controlled diabetes mellitus (36 subjects) with $HbA1c \geq 7\%$, 36 controlled diabetic patients ($HbA1c < 7\%$) and non-diabetics as the control group. All participants had health records in Diabetes Research Center. Informed consent was obtained from all volunteers. The inclusion criteria for the study group were having health records at the center, $HbA1c$ and fasting plasma glucose record for the previous three months and no eating, drinking or smoking for at least two hours before sampling. Participants who had consumed food during the previous two hours before sampling and subjects who had type 1 diabetes mellitus, gestational diabetes or other systemic diseases were excluded from the study. Duration of affliction with diabetes mellitus had to be less than five years and only patients taking metformin and glibenclamide were included in this study. Subjects in the control group were randomly selected from patients' family members who presented to the Diabetes Research Center and were matched in terms of age and sex.

Saliva collection:

The subjects were asked to sit quietly and vertically on the chair and collect their saliva

in their mouth for 30 seconds and then spit it all into sterile containers. Saliva samples were taken at 8-11 AM.

Measurement of saliva pH:

The PH of saliva samples was measured by a digital pH meter. The digital PH meter used in this study (AZ Company, Taiwan) was small, wireless and had high accuracy showing two decimal places with a margin of error of ± 0.05 , showing temperature when measuring the pH with high accuracy. The calibration points of this pH meter are at pH of 4, 7 and 10. Measurement of pH was done two times for each sample for accuracy and then the average of obtained numbers was recorded as the pH of the sample. In each electrode placement, we waited for 60 seconds and then the pH was read. After each use of the device, the electrode was cleaned and calibrated again by normal saline.

Viscosity measurement:

In order to measure the saliva movement in the capillary tube (in millimeters per second), we placed the tube in the saliva sample container for 10 seconds. The amount of saliva displacement in this tube was measured during 10 seconds. This test was repeated three times in order to reduce researcher error and the average of three times was recorded as the final salivary movement for each sample. Fasting plasma glucose and HbA1c of participants were extracted from patient records in the Diabetes Research Center.

All statistical analyses were carried out using SPSS version 20 (SPSS Inc., Chicago, IL, USA). ANOVA and Bonferroni multiple comparisons test were used. *P* value < 0.05 was considered statistically significant.

Results

In this study, 108 subjects in the age range of 30-70 years who were divided into three sex and age matched groups with 36 persons in each group (18 men and 18 women) were evaluated. The mean age was 54 ± 11.74 years in the control group, 53 ± 7.04 years in controlled diabetic patients and 55.56 ± 10.79 years in uncontrolled diabetics. This difference was not significant among the three groups ($P=0.492$). As there was no significant correlation between the pH and viscosity of saliva with age, there was no need to synchronize the three groups in terms of age, but covariance analysis was performed to control for age.

The mean (\pm standard deviation) salivary pH was 6.391 ± 0.45 in the control group, 6.169 cont in the controlled diabetic group, and 5.755 ± 0.54 in the uncontrolled diabetic group, respectively. *P*-values are shown in Table 1. Bonferroni test was applied for pairwise comparisons. Reduced pH in poorly controlled diabetic patients had a significant difference with that in the control group and controlled diabetic patients. Table 2 shows the results of saliva viscosity testing.

Table 1- Mean age, pH and rate of salivary movement in the capillary tube (viscosity) in the three groups

Variables	Age	Salivary movement in	pH
	(years)	capillary tube (mm)	
Groups	Mean \pm SD	Mean \pm SD	Mean \pm SD
Healthy control group (group 1)	54 \pm 11.740	5.78 \pm 2.537	6.391 \pm 0.45
Controlled diabetics group (HbA1c<7) (group2)	53 \pm 7.040	4.03 \pm 2.683	6.169 \pm 0.68
Uncontrolled diabetic group (HbA1c \geq 7) (group3)	55.56 \pm 10.79	3.39 \pm 2.057	5.755 \pm 0.54
P-value (ANOVA)	0.492	0.0001	0.0001

SD: Standard deviation

Table 2- Pairwise comparisons of pH and rate of salivary movement in the capillary tube (viscosity) using Bonferroni test

Groups	Variables	pH		Salivary movement in capillary tube (mm)	
		Mean Difference	P-value	Mean Difference	P-value
Healthy control group with controlled diabetic group (HbA1c<7)		0.2211	0.312	1.750	0.009*
healthy control group with uncontrolled diabetic group (HbA1c≥7)		0.6353	0.0001*	2.389	0.0001*
Controlled diabetic group with uncontrolled diabetics		0.4142	0.008*	0.639	0.808

Discussion

Diabetes mellitus is one of the most common metabolic diseases that has emerged as a dilemma in the health care community worldwide (11). In addition to micro-vascular and macro-vascular complications, this disease is capable of causing widespread effects on the oral cavity and teeth such as periodontal infection, gingival recession, tooth infections and tooth decay. Poor control of diabetes mellitus is an important risk factor for progression of complications (12).

The importance of saliva is well known in oral health (13). Saliva is vital for keeping and maintaining oral health. However, it gets little attention as long as it does not change in terms of quantity or quality. At present, saliva is studied as a helpful diagnostic method and many researchers have measured and investigated its physical and chemical properties in order to monitor systemic health, for diagnosis and also to control systemic diseases like diabetes mellitus since a close correlation exists between oral health and systemic health. The natural flow of the saliva is also important for oral health. Many studies have shown that low salivary flow is a risk factor for oral diseases (14).

One strength of the present study was that one of the target groups was controlled diabetic

patients. Impairments of salivary gland function may occur in controlled diabetics as well as uncontrolled diabetic patients (15), but no qualitative or quantitative study of saliva has been conducted comparing these two subtypes of type 2 diabetes mellitus. In this study, the salivary pH and viscosity in controlled diabetic patients, uncontrolled patients and healthy controls who had no other systemic diseases were evaluated. The results showed that there was a significant correlation between pH and viscosity of saliva with degree of hyperglycemia, so that the pH was reduced in poor controlled diabetics compared to healthy controls and controlled diabetic patients. However, saliva viscosity was higher in poorly controlled and well controlled diabetics compared to the healthy control group. Preoteasa et al. (16) showed that viscosity and salivary pH are two independent parameters and their changes had no relation to each other; the results of the current study were consistent with theirs but the mean age of patients in their study was higher than that in the present study. Puttaswamy et al. (17) reported a reduction in pH and salivary flow in patients with type 2 diabetes mellitus in comparison to healthy controls. Prathibha et al. (18) found significant changes in salivary flow rate and physical and chemical parameters between

diabetics and non-diabetic controls. It must be noted that the results of these three studies are consistent with our study.

Unlike our study, Collin et al, (19) in 1998 stated that diabetic patients were similar to healthy controls in terms of oral conditions such as flow rate, organic compounds, acidic bacteria of saliva and root and crown caries. This mismatch is due to different sample size in the study groups, passing 13 to 14 years since the diagnosis of type 2 diabetes mellitus and use of stimulated saliva. Bernadi et al, (20) in 2007 showed that metabolic control of hyperglycemia is not sufficient for improving salivary flow rate and salivary glucose concentration and there was no significant difference in pH and the buffering capacity of the saliva between diabetics and healthy controls, probably due to the use of stimulated saliva and also specific nutritional habits of the people residing in that region.

It is likely that decreasing the unstimulated salivary pH is due to the decrease in flow of un-stimulated saliva in diabetics in our study, because bicarbonate as the most important buffer is effective only in high flow rates of saliva and its concentration increases with an increase in salivary flow rate; however, the saliva flow of stimulated saliva is higher than that of unstimulated saliva, causing more alkalinity of the stimulated saliva. However, in hyperglycemia, several mechanisms cause acidic state of saliva. The first mechanism is that impaired metabolism of glucose and disruption of fat metabolism and production of large quantities of acetone and beta-hydroxybutyric acid acidify the saliva. Another suggested mechanism is increase in salivary microorganisms that play an important role in reaching the critical pH

(21,22). The saliva pH plays an important role in occurrence of tooth decay due to its buffering capacity. Cariogenic bacteria in acidic environments create tiny pores in the enamel surface which lead to cracks and porosities in the enamel surface and subsequently increased plaque accumulation and development of caries. Also, abrasion of lingual surfaces of the teeth is another deleterious effect of low saliva pH (23). Acidic pH, if continued for long-term in the oral cavity, leads to colonization of cariogenic bacteria instead of beneficial bacteria in the normal oral flora that could compromise oral health (22).

Increase in viscosity of unstimulated saliva in diabetics that is related to increase in concentration of salivary proteins (lactoferrin, lysozyme, albumin) and other inflammatory factors, can be attributed to the high incidence of gingivitis in diabetic patients. Increased viscosity of the saliva causes irritation and inflammation of the mucosa and reduces the washing property of the saliva, which is associated with other oral problems as diabetes mellitus progresses. Oral complications have deleterious effects on the quality of life.

This study showed that diabetes mellitus causes a reduction in saliva pH and increases the saliva viscosity especially if it is poorly controlled and both of these factors are risk factors for tooth decay and oral mucosal inflammation.

Conclusion

According to the findings of the current study, it seems that reduction in pH and increase in viscosity of the saliva are associated with type

2 diabetes mellitus especially in poorly controlled cases. As a result, prevention and early treatment of diabetes mellitus are necessary to prevent oral complications. In addition, oral health instruction and regular

oral examinations are necessary in patients with diabetes mellitus.

Conflict of interest: “None Declared”

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