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Evaluation of Unstimulated Salivary Flow and pH in Type I Diabetics Aged 6-16 years

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Abstract

Background: Diabetes mellitus Type I is the most common childhood metabolic disorder. There is evidence indicating that diabetics have different salivary flow and salivary compositions, as compared to non-diabetic individuals. This study investigated salivary flow and unstimulated salivary pH of Type I diabetics aged 6-16 years in comparison to the controls.

Methods: This analytical cross-sectional study was conducted on 120 children. Thirty children with Type I diabetes and ninety children as controls were matched with the diabetic group in terms of age and gender. Unstimulated salivary flow was collected by spitting method for 10 minutes and saliva pH was measured using a digital pH-meter. Salivary flow and pH were compared between two groups using chi-square and t-test.

Results: The mean salivary flow of diabetic and non-diabetic children was 0.268±0.168 and 0.454±0.307 mL/min, respectively. The mean pH of saliva of diabetic and non-diabetic children was 7.19±0.611 and 7.37±0.466, respectively. The mean unstimulated salivary flow was lower in diabetic children as compared to non-diabetic pediatric cases, and this difference was statistically significant (P=0.002). Although diabetic children had lower salivary pH compared to their healthy counterparts, the difference between the two was not statistically significant (P=0.10).

Conclusion: Diabetic children had lower mean unstimulated salivary flow, compared to non-diabetic children. Although diabetic children had a lower mean salivary pH than healthy children, this difference was not statistically significant.

Key Words: Child, Diabetes mellitus, Pediatric Diabetes, Saliva, Type I diabetes, Unstimulated Salivary Flow.

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1- INTRODUCTION

Diabetes mellitus is the most common chronic disease that leads to hyperglycemia. It is classified into two general categories: Type I, in which the beta cells of the pancreas lose their ability to produce insulin, and Type II, in which beta cells are defective or a decrease occurs in tissue sensitivity to insulin (1). Type I diabetes has affected more than half a million children around the globe (2). The prevalence and incidence of Type I diabetes in the world are increasing (3). Diabetes mellitus Type I is the most common childhood metabolic disease. Although this type of diabetes only accounts for 5-10% of all diagnosed types of diabetes, it is the main form of diabetes among children (4). The oral cavity is always exposed to saliva, the important role of which is to dilute and clean the mouth. Diabetes may cause changes in salivary glands causing low flow rate and other changes in saliva (5). This fluid is necessary to maintain oral health. The functions of saliva include the following: increasing the solubility of food, cleaning the mucous membranes of the oral cavity and teeth from food residues and debris, participating in many processes such as demineralization and remineralization, adjusting the attachment of microorganisms to teeth and other surfaces, and regulating and buffering the acid that is formed by bacteria during the digestion of food. The composition and function of saliva depend on its flow rate. When saliva flow increases, the concentration of sodium, calcium, chloride, bicarbonate, protein and buffering power increase (4). Salivary compounds are important factors in determining the prevalence of caries and oral health. Saliva maintains the integrity of oral tissue and controls the balance between remineralization and demineralization in a cariogenic environment. Besides, the salivary buffer can keep the pH constant in the dental plaque, thus preventing enamel demineralization (1). The oral cavity can be affected by diabetes, which may lead to various complications including dental caries, periodontal disease, oral mucosa disease, and impaired salivary function, which have significant effects on the quality of patients' life. Moreover, untreated oral diseases may increase the risk of poor metabolic control (1). Deficiency of insulin in diabetes may lead to lack of saliva and increased salivary glucose levels, which expose patients to an increased risk of caries (1). Diabetic patients have reported complaints of xerostomia and salivary dysfunction, which leads to decreased flow. Along with the decrease in saliva's buffering capacity, the risk of tooth decay and bacterial infection increases (1). Numerous studies have confirmed that patients with Type I diabetes mellitus experience decreased salivary flow. This may be attributed to insulin deficiency, which causes degenerative changes in the salivary glands in the form of intracellular lipid accumulation (4). There is evidence suggesting that diabetic patients have different salivary flow and salivary composition compared to non-diabetic subjects; however, the results of the articles involve discrepancies; some have reported that both stimulated and unstimulated saliva is reduced in diabetic patients, while others have reported that only unstimulated salivary flow decreases (6).Thus, it is mandatory to investigate the possible correlation between diabetes and salivary changes. The purpose of this study was to investigate salivary flow and unstimulated salivary pH in children with Type I diabetes aged 6-16 years, in comparison to healthy controls.

2- METHODOLOGY

2-1. Subject

In this analytical cross-sectional study, the investigated sample consisted of 30

children with insulin-dependent diabetes aged 6-16 years for whom at least one year had passed since the onset of their disease, and 90 non-diabetic people as a control group after matching for age and gender. A total of 300 recorded files of the patients with Type I diabetes were assessed and 42 patients who were qualified for inclusion entered the study after signing informed written consent. Also, 12 patients were excluded from the study due to their lack of cooperation in collecting saliva samples.

2-2. Ethical considerations

This study has been approved by the "Committee of Ethics in Human Research" at Shahid Sadoughi University of Medical Sciences, Yazd, Iran, with the ethics code of No. IR.SSU.REC.1400.019. Diabetic cases, eligible for the study, were selected from the patients referred to Yazd diabetes center, and the participants in the control group were randomly selected from the schools of Yazd city. Sampling was done during 3 months from October 2021 to January 2022.The research method, topic, and objectives were explained to all patients. After obtaining informed written consent, the patients were included in the project.

Before sampling, the participants were urged to refrain from eating, drinking, chewing gum, brushing teeth, and any act that stimulates saliva for at least 1 h before saliva collection.

The saliva sampling container was made of plastic and sterilized. The size of these containers was chosen so that saliva could be collected and the probe of the pH-meter could be immersed in the saliva sample. To minimize the effects of the circadian rhythm on the flow and composition of saliva (7), all saliva samples were collected during 8-10 a.m. (4). The subjects were asked to sit upright in a chair for 10 mins without stress and collect saliva while their head was slightly bent forward (8). Saliva collection was done without any stimulation for 10 min by spitting method. Then, the mass of saliva was measured with the electronic scales NOTEBOOK (made in China, model 1108-5, with an accuracy of 0.01) in such a way that the mass of the container for collecting saliva was measured and noted by the scale, and then it was measured again after sampling, and the difference between the obtained numbers was recorded. Based on the density of saliva which is 1 g/mL (1 g=1 mL) (9), the mass of saliva was an indicator of the volume of saliva. The amount of unstimulated salivary flow rate (USF) was calculated in mL/min through the following formula:

$$\mathrm{USF} = \frac{\text{collected salivary volume (mL)}}{\text{Time of saliva collection (min)}}$$

After collecting the saliva samples, saliva pH was measured immediately to avoid time-dependent pH changes or loss of CO_2 (6). Analysis was performed with AZ digital pH-meter (Model 86502, made in Taiwan) at a temperature of 25°C. Some of the features of this device include the high accuracy that shows up to two decimal places with an accuracy of 0.02, as well as showing the temperature at the time of pH measurement; the calibration solutions of this device have pHs of 7, 4, and 10. To check the pH of the samples, the device was first calibrated. The calibrated solution used in this study had a pH of 7.

2-3. Inclusion and exclusion criteria

Inclusion criteria for participants in the diabetic group were: at least one-year history of the diagnosis of diabetes, good general health regardless of diabetes status, and absence of proteinuria or affliction with other diseases such as thyroid and celiac disease. The subjects in the control group were supposed not to have underlying diseases and orthodontic devices. Additionally, if the subjects were non-cooperative in collecting saliva and

did not return at the designated time for sampling, they were excluded from the study.

2-4. Statistical analysis

After collecting and controlling the data, they were analyzed with SPSS17. Chi-square and t-test were used for statistical comparisons. The significance level was considered at 0.05. The required tables and indexes were prepared.

3- RESULTS

In this study, 120 people aged 6-16 years were examined in two groups: participants with Type I diabetes (30 people) were assigned into the case group and 90 children were selected as healthy controls after matching for age and gender. In this study, the mean ages of the diabetic group and the control group were 12.07±3.02 and 11.78±2.54 years, respectively (P>0.05). The diabetic group included 50% girls and 50% boys, and the control group included 51.1% girls and 48.9% boys. There were no statistically significant differences in age and gender between the two groups of diabetics and controls (P>0.05). Furthermore, the mean salivary flows were 0.268±0.168 and 0.454±0.307 mL/min in diabetic subjects and in non-diabetic groups, respectively; the mean salivary flow in diabetic subjects was significantly lower than that of non-diabetic subjects (**Table 1**).

Table-1: Mean salivary flow (mL/min) in the two diabetic and healthy groups

Group	No. of sample	Mean ± SD of salivary flow	CI 95% Upper limit	Lower limit	Minimum	Maximum	p-value
Diabetics	30	0.268±0.168	0.331	0.206	0.07	0.80	0.002
Non-diabetics	90	0.454±0.307	0.519	0.390	0.10	1.80	

As shown in **Table 2**, the mean pH of saliva was 7.19±0.611 and 7.37±0.466 in diabetic and non-diabetic groups, respectively, and there was no significant difference between the two groups.

Table-2: Mean pH of saliva in two diabetic and non-diabetic groups

Group	No. of sample	Mean ± SD of salivary pH	CI 95% Upper limit	Lower limit	Minimum	Maximum	p-value
Diabetics	30	7.19±0.611	7.42	6.96	6.13	8.56	0.10
Non-diabetics	90	7.37±0.466	7.46	7.27	5.88	8.24	

Moreover, the mean saliva flow was 0.225±0.148 and 0.335±0.208 mL/min in the diabetic and non-diabetic girls, respectively, with no significant difference (P=0.064). The mean salivary flow rates were 0.311±0.180 and 0.580±0.343 mL/min in diabetic and non-diabetic boys, respectively, which were significantly different (P=0.006). The means and standard deviations of saliva pH were 7.31±0.643 and 7.26±0.556 in diabetic and non-diabetic girls, respectively, which were not significantly different (P=0.76). The mean pH of saliva was 7.0±0.573 and 7.48±0.318 in diabetic and non-diabetic boys, respectively, with a statistically significant difference (P=0.001). The mean HbA1c of diabetic patients was 9.36%±1.92% with a range of changes between 5.7% and 13.3% with a sample volume of 30 patients. The Pearson correlation coefficient between HbA1c and salivary flow was 0.132, meaning that there was a direct and weak correlation

between HbA1c and salivary flow (P=0.496). The Pearson correlation coefficient between HbA1c and pH of saliva was -0.068, suggesting that there was an inverse and weak correlation between HbA1c and pH of saliva (P=0.72). The mean salivary flow was 0.246±0.156 and 0.276±0.175 mL/min in the group and in the experimental cases, respectively, which was not statistically significant (P=0.67).

Besides, the mean pH of saliva was 7.10±0.394 and 7.22±0.678 in the control group and in the experimental group, respectively, which were not significantly different (P=0.65).

4- DISCUSSION

Given that previous studies have proven that the effect of saliva on dental caries is mainly due to unstimulated salivary flow and composition of saliva (10), in this study, unstimulated saliva was investigated. Various methods such as paper pH-meter and digital pH-meter have been previously used by researchers. Due to the fact that pH paper cannot show the numbers accurately and has low accuracy, the method of pH-meter devices was used. The advantages of this device are high accuracy (0.02) along with its displaying the temperature of the test environment that minimizes the possibility of work error caused by the difference in ambient temperature. The results of the present study demonstrated that the mean unstimulated salivary flow of the group of diabetic patients was lower compared to the group of healthy children, and this difference was statistically significant; this is consistent with some studies (1, 5, 6, 8, 11-17). In the study by Ferizi et al., diabetic children had less stimulated saliva flow compared to the control group, and they considered the cause of decreased saliva to be hyperglycemia and glycosuria, which lead to lower saliva flow; the difference with the present study is the measurement of stimulated saliva (1). The results of the present study are not consistent with Malicka's study. It should be noted that in this study, salivary flow was investigated in patients with Type II diabetes mellitus while they were resting (18). The difference in the results can be attributed to the difference in saliva collection methods (stimulated or unstimulated), the time of saliva collection, the condition and position of the patient during collection, and the lack of age and gender matching between the case and control groups .In general, the cause of decreased salivary flow in diabetics can be due to hyperglycemia, glycosuria, and dysfunction of the parasympathetic and sympathetic nervous system in diabetic patients, which is a form of peripheral neuropathy. Excreting more body fluids, which subsequently results in reduced salivary secretion, are pathological changes in the structure of salivary glands and microvascular disorders. The results of the present study showed that the mean pH of saliva in the diabetic group was lower compared to the healthy children, but this difference was not statistically significant; this is consistent with the findings of some other studies (11, 19-22). The results of the present study in terms of pH are not consistent with the studies by Basir (8) and Stetiu (16). It should be pointed out that in these two studies, a paper strip was used to measure pH, which is less accurate than a digital pH-meter. In the present study, the Pearson correlation coefficient between flow and pH of saliva was 0.06, meaning that there is a direct and weak relationship between flow rate and pH of saliva. Since almost 85% of unstimulated saliva is the result of the secretion of the submandibular and parotid glands, and the pH of saliva secreted from these two glands does not change much with the decrease or increase in the flow rate, other factors also affect the pH of saliva. For example, Kjellman has suggested that dietary recommendations may affect

buffering capacity (23). Among the factors that may affect the pH of saliva, we can mention the pH of plasma and the amount of acidogenic bacteria in the oral cavity. It is not possible to consider only the flow to exert an effect on the pH of saliva; it can be stated that since diabetic children have a lower saliva flow than healthy people, they have lower saliva pH. The findings of the present study, in line with some previous studies (13, 24-26), revealed that there is a direct and weak correlation between HbA1c and non-stimulated salivary flow. According to Zachariasen(11), diabetes affects the structural and functional integrity of salivary glands; hence, glycemic control is not efficient to return the salivary flow to normal. In the study by Carneiro et al. with the aim of exploring the effect of glycemic control on the oral health of Type I diabetic children and adolescents, they observed a significant decrease in the flow of stimulated saliva along with an increase in HbA1c; this is not consistent with the results of the present study (27). Consistent with the literature (11, 26, 28), the results of the present study further suggested that there is an inverse and weak correlation between HbA1c and salivary pH. Reuterving et al. reported that there is no significant difference in pH and buffering capacity of saliva based on metabolic control, and the level of metabolic control of diabetes does not appear to be of great importance for salivary flow (stimulation) and its components except saliva glucose concentration (28). Finally, Bernardi et al. reported that there was no significant difference in saliva pH in well-controlled and poorly controlled subjects; this is similar to the results of the present study (11).

4-1. Limitations of the Study

Limitations of this study include small sample volume, limited geographical area, and the cross-sectional design.

5- CONCLUSION

In the present study, the mean flow of unstimulated saliva in diabetic group was lower compared to that of the healthy controls. Although the mean pH of saliva in diabetic patients was lower in comparison to the control group, this difference was not statistically significant. It is suggested that the flow and pH of stimulated saliva and other compositional changes of saliva be investigated in future studies.

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