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Evaluation Chemical Composition of Unstimulated Saliva, in Patients with Type I Diabetes Mellitus

Masoomeh Shirzaii,[*1] Fathemeh Heidari[2]

1. Department of Oral and Maxillofacial Surgeon, Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran
2. Department of Pathology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

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*Corresponding author at:
Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran.
E-mail:
shirzaiy@gmail.com

Abstract

***Background*:** Diabetes will result in change in qualitative and quantitative function of saliva. The purpose of the study was to determine the chemical composition (combination) of unstimulated saliva in patients with type I diabetes mellitus.

***Materials and Methods*:** In this case-control study, unstimulated saliva of 25 patients with type I controlled diabetes (20-30 years) and 25 healthy person who matched with the case group in respect of age and gender was gathered and analyzed in order to evaluate the chemical composition of saliva. The data was analyzed using SPSS-18 and independent *t*-test.

***Results*:** Salivary Ca^{2+} concentration in diabetic patients was equivalent to 9.2±2.3 mg/dl and in healthy individuals was 9.4±0.7 mg/dl; Sodium level (Na^{+}) in diabetics was equal to 1.3±11.8 mg/dl and in healthy individuals was 9.9±2.5 mg/dl; Potassium level (K^{+}) in diabetics was equal to 19.5±6.3 mg/dl and in healthy individuals was 15.8±4.4 mg/dl; Urea level in diabetics was equal to 19±3.8 mg/dl and in healthy individuals 9.7±1.4 mg/dl; and Phosphorus level (P^{+}) in diabetics was equivalent to 12.7±4.6 mg/dl and in healthy individuals was 11±4.8 mg/dl. Salivary K^{+}, Urea, and Na^{+} concentration in both groups was significantly different (p=0.05).

***Conclusion*:** Chemical composition of saliva in diabetics in relation to healthy individuals was different; Urea and Potassium level increased and Sodium level decreased.

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Introduction

One of the important issues which have received attention nowadays is the effect of saliva performance on oral cavity hygiene [1]. It is difficult to evaluate saliva performance due to the high varieties (diversities) in this substance compared to serum that with studying its existing elements, physiological and pathological conditions can be easily distinguished [2]. Saliva collection in large populations for very important diagnostic purposes is easier than serum [3].

It is very important to define the normal limit (range) of salivary elements so that cholistin can detect all the salivary disorders associated with pathological conditions [2]. Diabetes is the most common metabolic disease associated with salivary hypofunction with increased susceptibility to oral infections such as caries or periodontitis. In the study of Ben-Aryeh et al., it was specified that salivary secretion in diabetic patients will change both quantitatively and qualitatively [4]. In the study of Dodds et al., there was no significant difference in the chemical composition and flow rate of stimulated and unstimulated saliva of diabetic patients and the healthy group, but salivary amylase activity was significantly higher in diabetics [5]. In the study of Lopez et al., glucose, urea and total protein increased in the diabetic patients, while calcium values were decreased [6]. In diabetic patients, salivary gland secretions are subjected to qualitative and quantitative change and finally lead to oral hard and soft tissue injuries. These changes will increase caries and periodontal problems [2, 7]. In the study of Mata et al., salivary composition in diabetic patients compared to the healthy individuals had significant differences. So that the amount of calcium (Ca^{2+}) had increased and the amount of zinc had decreased [2]. IgA، IgG، Ca^{2+} and K^{+} level of Salivary in diabetic patients will increase significantly [8-9]. There is few studies about changes in the chemical composition of saliva in controlled diabetic patients in Iran. So in the present study, chemical quality of stimulated and unstimulated saliva in controlled diabetes type I was evaluated.

Materials and Methods

In this analytical and case-control study, 25 patients with controlled diabetes type I (20-30 years) who had the qualifications for entering the study were selected randomly among the diabetic patients who referred to Diabetes Center of Ali-Asghar Hospital as a case group. HbA_1c criterion (< 8) was used to determine the level of disease control. The qualifications include: having diabetes for at least 2 years, not using medicines (drug), no affliction to chronic diseases and their complications

(diagnosed by a specialist), no smoking, drugs and alcohol.

Table 1. Chemical composition of saliva in diabetes mellitus type I patients and control group

Chemical composition of saliva	Diabetes mellitus type I patients			Healthy			*p*-Value *
	Mean±SD (mg/dl)	Min	Max	Mean±SD (mg/dl)	Min	Max	
Ca^{2+}	9.2±2.3	7.6	20	9.4±0.7	8.2	11.4	0.5
Urea	19±3.8	5	80	9.7±1.4	5	48	0.03
P^{3+}	12.7±4.6	6.6	24	11±4.8	2.8	24	0.1
K^{+}	19.5±6.3	11	31	15.8±4.4	2	26	0.01
Na^{+}	1.3±11.8	9.7	6.1	9.9±2.5	5.2	11.5	0.02

*: Independent *t*-test

Table 2. Chemical composition of saliva in diabetes mellitus type I patients and control group by sex

Subjects (sex)		Number	Mean±SD (mg/dl)	*p*-Value
Ca^{2+} (Diabetes mellitus)	Male	8	10.3±3.9	0.5
	Female	17	8.7±0.6	
Ca^{2+} (Healthy)	Male	8	9.4±0.8	< 0.001
	Female	17	9.4±0.7	
Urea (Diabetes mellitus)	Male	8	44.7±23.5	0.01
	Female	17	21.3±10.7	
Urea (Healthy)	Male	8	17.5±6.1	0.8
	Female	17	20.5±10.7	
P^{3+} (Diabetes mellitus)	Male	8	16.2±5.6	0.04
	Female	17	11.05±5.01	
P^{3+} (Healthy)	Male	8	10.8±4.5	1
	Female	17	11.05±5.03	
K^{+} (Diabetes mellitus)	Male	8	22.4±8.2	0.02
	Female	17	18.1±6.8	
K^{+} (Healthy)	Male	8	14.2±4.06	0.2
	Female	17	16.5±4.5	
Na^{+} (Diabetes mellitus)	Male	8	10.09±1.3	0.8
	Female	17	12.8±1.4	
Na^{+} (Healthy)	Male	8	7.5±1.01	0.1
	Female	17	2.5±0.9	

The patients were also examined for oral pathological conditions and medical problems (like radiotherapy, Sjogren's syndrome) which affect their salivary glands and its secretions. Age, sex, diabetes type, control method, average of glucose levels and history of smoking and drugs were obtained by using the patient's medical record and interview with him/her.

The control group included the healthy individuals who had no smoking, drugs and alcohol record as well as drug therapy, oral or systemic diseases within the past three months and had at least 2 normal fasting blood glucose tests and were matched with the case group for age and sex.

All the patients had a written letter of consent and they were excluded from the study in case of unwillingness for cooperation. After completing the questionnaire, unstimulated saliva of the individuals was collected in the morning and the patients were asked not to eat and drink 90 minutes before testing in order to prevent the irritation of saliva secretion. Spitting was used for collecting saliva. The patient ejected his saliva (spat) into a tube with a certain weigh every 60 seconds for 2-5 minutes. Then, the collected saliva was kept in a centrifuge with 10000rpm at 4°C for 5 minutes in order that the centrifuged cellular and bacterial debris be separated and so further analyses to be conducted at 80°C.

In this examination, sodium, potassium, calcium, urea and inorganic phosphorus level was calculated for both groups (according to mg/dl). Salivary calcium was measured using colorimetric method and calcium kit (Iran biochemistry). Salivary phosphorus was measured using molybdate phosphorus or colorimetric method and inorganic phosphorus kit (Iran biochemistry) and absorbance of the standard and test against reagent blank was read with a spectrophotometer. Salivary urea was determined by diacetyl monoxime method and absorbance of the Standard and Test against Reagent Blank was read with a spectrophotometer. Sodium and potassium were measured by a flame photometer. Finally, the data was analyzed using SPSS-18 and independent *t*-test.

Results

In the present study, the concentration of unstimulated salivary calcium in diabetic and healthy individuals was 9.2±2.3 and 9.4±0.7, respectively. Chemical composition of saliva in the case and control groups is presented in table 1. Calcium and phosphorous level of saliva in the two groups was not significantly different, but the level of potassium and urea in diabetic patients was significantly more and sodium level was less ($p< 0.05$) (Table 1). The average concentration of urea, potassium and phosphates in diabetic men was significantly more than diabetic women ($p< 0.05$) and in the control group the level of calcium in men was more than women ($p< 0.05$). The chemical composition of saliva is presented in table 2 by sex.

Discussion

In this study, the quality of unstimulated saliva was compared in patients with controlled diabetes type I and healthy individuals. The results indicated that chemical composition of saliva is different in both groups. Disorder in the function of salivary glands in diabetic patients is due to diabetic neuropathy and change in Salivary gland parenchyma. Changes of salivary gland parenchyma and autoimmune lymphocytic infiltration which occur in this disease are nearly similar to changes which occur in pancreatic gland parenchyma (islets of Langerhans) and are involved in pathogenesis of diabetes [2, 7, 10].

These changes and disorder are more observed in the function of salivary glands in uncontrolled diabetic patients [2, 11, 12]. Although in the recent study, the controlled diabetic patients were examined.

Subjects of the study were controlled diabetic patients. Thus, disorder in the function of salivary glands is not restricted to poorly controlled diabetes and the condition may also occur in controlled diabetes. Between the two types of diabetes (controlled and poorly controlled), no quantitative comparison has been conducted, yet [2, 13].

In the present study, calcium (Ca^{2+}) level of saliva in diabetic and healthy individuals was almost the same, being consistent with the results of the study of Reuterwing [14]. But in the study of Lopez et al. and Moreira et al., concentration of Ca^{2+} in diabetic patients has been decreased [6, 13]. Differences in the age group of the subjects of study could be the reason. In the study of Lopez et al. diabetic children were examined [6]. But in the present study, the age group of 20-30 years was examined.

In the study of Mata et al., the level of salivary calcium (Ca^{2+}) in diabetic patients had been increased significantly [2]. The increase of salivary calcium in diabetic patients is the result of reduction in saliva output and increase in protein concentration. The increase of salivary calcium will increase the amount of plaque formation and the risk of periodontitis [8, 9].The increase of caries (decay) and periodontal disease in diabetic patients is the result of reduction in saliva output. In the study of Ben-Aryeh et al., there was not a certain relationship between the parameters of saliva and caries. However, salivary proteins (albumin, lysozyme and lactoferrin) had direct relationship with the amount of gingivitis (GI index) [4].

In the present study, concentration of salivary potassium (K^{+}) in diabetic patients was significantly more than healthy individuals. The amount of saliva secretion in diabetic patients had been decreased significantly and the secreted saliva has a relatively high viscosity. With reduction in saliva output, the amount of existing ions in saliva will change significantly [2, 12, 13].

In the study of Ben-Aryeh et al., the level of stimulated and unstimulated salivary potassium in diabetic patients was more than healthy individuals [4]. However, in the study of Mata et al. the level of K^{+} in diabetic patients has been deceased significantly [2].

In the present study, concentration of salivary sodium (Na^{+}) has been decreased significantly. However, in the study of Ben-Aryeh et al. and Mata et al., the level of salivary Na^{+} in diabetic patients was not significantly different with the control group [2, 4]. In the present study, the concentration of unstimulated salivary urea in diabetic patients was more which is consistent with the results of the study of Moreira et al. in which salivary urea was studied through enzymatic method [13].

In the present study, the level of unstimulated salivary P^{+} in diabetic patients was more but the difference was not statistically significant, being consistent with the obtained results in the study of Reuterwing et al. in this regard [13]. However, it is not consistent with the results of the study of Ben-Aryeh et al. It seems that besides systemic condition, type of nutrition, race and genetics have a significant role in the quality and quantity of saliva, as well [2].

In the present study, chemical analysis of saliva by sex indicated that the level of salivary phosphorous and potassium in diabetic men is significantly more than diabetic women, while concentration difference of salivary phosphorous and potassium in the women of case and control group was not statistically significant. Likewise, the level of salivary calcium in diabetic and healthy women was significantly different and it was more in diabetic women but the concentration of salivary calcium in the men was not significantly different. While in the study of Lopez et al. the level of salivary calcium in diabetic men was less than diabetic women. But, the level of this marker in the males and females of the control group was nearly the same and also the level of salivary urea in the males of the case and control group was more than females [6]. The current study and the study of Lopez et al. are one of the few studies which presented the chemical composition of saliva in men and women separately. In other researches, the difference of salivary markers has not been studied in the two sexes. But, this study indicated that these markers can also be significantly different in men and women.

Diabetes will result in change in qualitative and quantitative function of saliva. Change in chemical composition of salvia is not restricted to uncontrolled diabetic patients and the status is also observed in controlled diabetic patients. In the present study, chemical composition of unstimulated saliva was evaluated, although chemical analysis of the stimulated saliva will also provides us with useful information. Comparison of the saliva rate and composition in controlled and uncontrolled diabetes in further studies can be an influential guide in determining the pathogenesis of diabetes oral manifestations (complications).

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest
The authors declare no conflict of interest.

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