RESEARCH ARTICLE

Salivary Profile in Adult Type 2 Diabetes Mellitus Patients: A Case-control Study

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Abstract

Objective: To evaluate salivary factors in type 2 diabetes melliuts patients.

Methods: The case-control study was conducted from June to November 2016 at Kermanshah University of Medical Sciences, Kermanshah, Iran, and comprised patients with type 2 diabetes mellitus and healthy controls matched in terms of age and gender. Unstimulated saliva samples were collected in the morning after an overnight fast of 8-12 hours. The samples were centrifuged at 1500 rpm for 5 minutes, and every isolated transparent liquid was immediately frozen at a temperature of -45°C in a tube. The test sample was later aspirated, and readings were taken. The data was analyzed using SPSS 16.

Results: Of the 200 subjects, 100(50%) were diabetic patients and 100(50%) were healthy controls. The two groups were matched with regard to age, gender, diabetes duration, serum glucose, and glycated haemoglobin ($p>0.05$ each). In terms of laboratory variables, there were significant differences between the groups related to urea, phosphorus, pH, and glucose ($p<0.05$ each). Urea and glucose levels were higher in the patient group than the controls ($p<0.05$ each). Also, calcium and total protein levels were higher in male patients compared to female patients ($p<0.05$ each).

Conclusion: Salivary pH, urea, calcium, phosphorus, glucose, and total protein levels could be biochemical parameters for screening, diagnosis and monitoring of diabetes.

Key Words: Diabetes mellitus, Type 2, Saliva, Glucose, Calcium. (JPMA 69: 190; 2019)

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases affecting approximately 8.4% adults aged 18-99 years in 2017 and predicted to rise to 9.9% in 2045.[1] This disease is a public health problem with important social repercussions.[2] It is a relatively common ill-health condition in Iran with a prevalence of 7.7% in the age range of 25-64 years and with a rather high burden.[3] Diabetes can be divided into types 1 and 2. In type 1 diabetes, the β-cells of the pancreas are destroyed by the autoimmune mechanism. In type 2 diabetes mellitus (T2DM), a resistance to insulin is developed.[4] A common oral problem associated with DM is xerostomia,[5-8] whose intensity is significantly correlated with salivary level of glucose,[5] increased infections, salivary dysfunction, dental caries[6-8] and lichenoid reaction caused by certain anti-diabetic drugs[9] in DM patients. Differences in saliva production and composition have been observed previously between diabetic and non-diabetic subjects.[10] Approximately 5% of all patients visiting dental clinics are reported to have diabetes.[11] Therefore, examination of the composition of saliva in patients with diabetes may be useful to understand why oral manifestations occur and how they should be treated.[5] Several salivary factors have been reported to affect the incidence of dental caries in T2DM patients.[12] There is no consensus on the possible association between T2DM and salivary dysfunction in diabetes.[13] The current study was planned to assess the salivary factors of DM patients as well as the impact of gender on these factors.

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Materials and Methods

The case-control study was conducted from June to November 2016 at Kermanshah University of Medical Sciences, Kermanshah, Iran, and comprised T2DM

patients and healthy controls matched in terms of age and gender. After approval from the institutional ethics committee, the sample size was determined for which a pilot study was conducted comprising 20 T2DM patients and 20 controls. Their mean of potential of hydrogen (pH) was measured which considered a major salivary marker and an inexpensive factor. Alpha and Power were 0.05 and 0.80, respectively. Using Stata software to compare the mean values between the groups, the sample size was estimated to be 100 participants in each group.

The patients were selected from the Diabetes Research Centre of the university, while the healthy controls were selected from the Medical Centre. Written informed consent was obtained from all the subjects. The inclusion criteria consisted of laboratory tests for glycated haemoglobin (HbA1c) and fasting blood sugar (FBS) to separate patients from controls. Each test was done twice. Those with HbA1c $\geq$ 6.5% or FBS$\geq$126 mg/dl were declared diabetic patients. The patients had a history of diabetes for a minimum two years. Both the patients and the controls were aged 30-70 years with no history of addiction, alcohol consumption, fatty liver, cancer, hepatitis, human immunodeficiency virus (HIV), infection, hypothyroidism, hyperthyroidism or oral diseases. The controls had no history of diabetes. Subjects like pregnant women, patients consuming oral medications except for diabetic drugs, and controls consuming oral medications were excluded.

Un-stimulated saliva samples were collected in the morning between 8am and 10am. A total of 15ml saliva was taken within 5 minutes, while the subjects were asked not to eat, smoke or drink (except water) over the night prior to the collection of the samples. Before sampling, all subjects had to thoroughly wash their mouths, and after several minutes, they started to collect saliva. Then, 2ml of un-stimulated salivary sample was collected from every subject and the samples were centrifuged at 1500 rpm for 5 minutes, and every isolated transparent liquid was immediately frozen at a temperature of -45^{o}C in a tube.

The salivary tests were performed on every sample using German-made ErbaMannheimXL600 analyser (Pars Azmon Co., Iran), by photometric method. The saliva sample in the tube was separated and placed in three other tubes labelled 'blank', 'standard' and 'test'. Then, 1000 µl of reagent solution was pipetted into all the three tubes. Next, 10µl of standard was added to the test tube marked as 'standard' and 'test'. After complete mixing, the test tubes were kept at 370C for 10 minutes in an incubator before aspiration. The reagent 'blank' was aspirated in the analyser first, followed by standard solution, and the readings were noted. Finally, the test sample was aspirated, and readings were noted again. Salivary pH was measured by HI98107 pH-meter (Hanna Co., Italy) with an accuracy of ±0.2 (25°C/77°F).

Data was analysed using SPSS 16. Independent t-test was used for comparison of means, and chi-square test was used for comparison of genders in the two groups. Results were presented as mean±SD. Scatter plot and regression line were drawn to determine the correlation of salivary glucose with serum glucose and HbA1c by Pearson correlation coefficient. P<0.05 was considered statistically significant.

Results

Of the 200 subjects, 100(50%) were diabetic patients and 100(50%) were healthy controls. The two groups were matched with regard to age and gender. Diabetes duration, serum glucose, and glycated haemoglobin (p>0.05 each) (Table 1). In terms of laboratory variables, there were significant differences between the groups related to urea, phosphorus, pH, and glucose (p<0.05 each). Urea and glucose levels were higher in the patient group than the controls (p<0.05 each) (Table 2). Also, calcium and total protein levels were higher in male patients compared to female patients (p<0.05 each) (Table 3).

Table-1: Baseline characteristics of study subjects (n=100).

Variables	Controls	Patients	*p*-value
Mean Age (years)	55±9.3	52.7±8.5	0.08
Range	32-68	61-75	
Sex			
Male/Female	46/54	42/58	0.60
Mean Diabetes duration (years)	-	9.6±6.6	-
Range	-	2-30	
Mean Serum glucose, mg/dL	-	161±59.7	-
Range	-	71-372	
Mean Serum HbA1c, %	-	8.6±1.6	-
Range	-	6-13.2	

Table-2: The comparison of laboratory variables in both groups (n=100).

Variables	Controls	Patients	p-value
Urea, mg/dL			
Mean±SD	29.28±13.17	34.16±20.71	0.048
Range	31-64	3-141	
Calcium, mg/dL			
Mean±SD	2.15±1.67	1.80±2.29	0.220
Range	0.3-11.6	0.05-13.1	
Phosphorus, mg/dL			
Mean±SD	19.59±12.62	12.83±7.84	<0.001
Range	0.6-104.7	0.3-37.2	
Total Protein, mg/dL			
Mean±SD	0.26±0.37	0.21±0.37	0.328
Range	0.01-2.11	0.01-2.13	
pH			
Mean±SD	7.08±0.25	6.97±0.25	0.002
Range	6.39-7.42	6.31-7.41	
Glucose, mg/dL			
Mean±SD	2.67±2.80	5.63±3.18	<0.001
Range	0.03-21	2.1-21	

SD: Standard deviation.

There were no significant correlations between salivary glucose and serum glucose and HbA1c levels (p>0.05 each) (Figures 1-2).

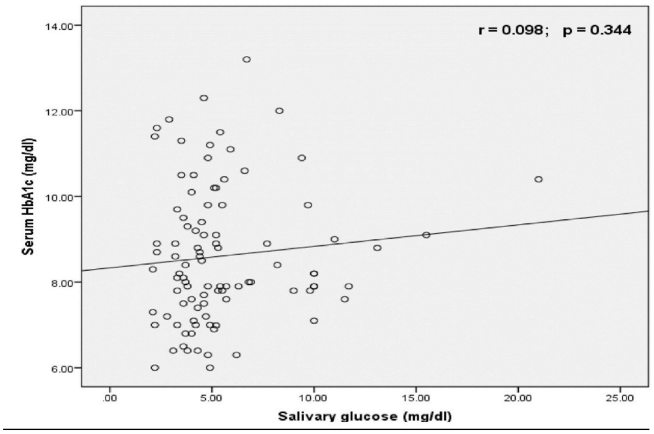

Figure-1: Scatter plot and regression line of salivary glucose levels and serum glucose levels in the diabetic patients.

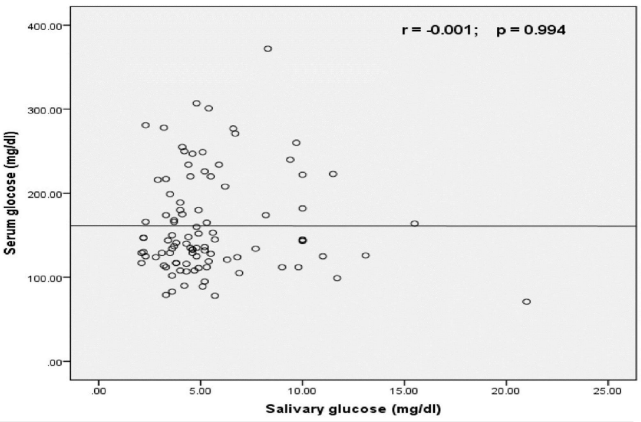

Figure-2: Scatter plot and regression line of salivary glucose levels and serum glycated haemoglobin (HbA1c) levels in the diabetic patients.

Table-3: The comparison of laboratory variables in both groups based on gender.

Variables	Controls		*p*-value	Patients		*p*-value
	Male(n=46)	Female (n=54)		Male (n=42)	Female (n=58)	
Urea, mg/dL						
Mean±SD	29.58±13.23	29.02±13.22	0.836	36.04±21.78	32.78±19.97	0.441
Range	9-64	3.1-63	0.6-89	0.3-141		
Calcium, mg/dL						
Mean±SD	2.28±1.54	2.02±1.76	0.437	2.45±3.22	1.32±1.04	0.014
Range	0.3-5.5	0.4-11.6	0.05-13.1	0.2-4.8		
Phosphorus, mg/dL						
Mean±SD	19.62±15.32	19.55±9.90	0.981	11.36±6.72	13.89±8.44	0.111
Range	0.6-39.5	3.5-104.7	1.9-27.9	0.3-37.2		
Total Protein, mg/dL						
Mean±SD	0.29±0.33	0.23±0.39	0.434	0.31±0.51	0.12±0.20	0.011
Range	0.01-1.2	0.01-2.11	0.01-2.13	0.01-1.47		
pH						
Mean±SD	7.04±0.28	7.10±0.21	0.216	6.97±0.26	6.96±0.24	0.850
Range	6.39-7.41	6.49-7.42	6.31-7.41	6.48-7.38		
Glucose, mg/dL						
Mean±SD	2.77±2.76	2.57±2.84	0.721	5.94±3.88	5.40±2.55	0.401
Range	0.2-15.5	2.1-21	0.03-21	2.1-15.5		

SD: Standard deviation.

Discussion

The results of evaluation of salivary profile in T2DM patients compared with healthy controls showed that urea and glucose levels in the patients were significantly higher and phosphorus level and pH were significantly lower in the patients than the controls. The comparison of serum urea values with salivary urea values showed that in 30 diabetic patients vs. 30 adult healthy controls (age- and gender-matched), serum urea values were significantly higher than salivary urea values, and salivary urea levels were higher in diabetic patients than in healthy controls.[14] One study included 60 participants of both genders with age range of 30-70 years (30 insulin-dependent diabetic patients and 30 controls not suffering from diabetes), in which salivary urea and salivary glucose levels were significantly higher in diabetic patients than in control group.[5] In other studies[15,16] and the present study, similar results were reported for glucose concentration. The present study also confirmed the above-mentioned results about salivary urea level.

In Brazil, 88 diabetic and 39 non-diabetic adults were included in a study, in which glucose, urea and calcium levels were significantly higher in diabetic patients, whereas total protein was lower in the patients compared to the controls.[2] Similar[17,18] and contradictory[12,19] results have been reported for calcium levels. It has also been found that the diabetic patients have an increased resting and stimulated salivary protein concentration compared to healthy participants.[18] A study[20] showed a significant correlation between DM patients and healthy subjects in salivary glucose, amylase, calcium and phosphorous. Salivary phosphate was found to be significantly lower in patients than in controls.[12]

One case-control study13 comprising 30 healthy controls and 30 T2DM patients reported that salivary pH and calcium level were significantly lower in patients than controls. Moreover, significantly higher levels of salivary glucose and total proteins were reported in patients than in controls.[13] Other studies have shown that DM patients present a decrease in pH.[6,19]

One study[21] on 60 patients aged 25-50 years with known T2DM and with decayed, missing and filled teeth (DMFT) index >10 and 60 non-diabetic (caries-active and age- and gender-matched) subjects showed that salivary calcium ions were significantly higher in non-diabetic individuals. A study[22] reported that the calcium content of saliva was low in caries-active persons, confirming our findings that the state of calcium in saliva may be related to dental caries' risk. Diabetic patients had a higher DMFT index, which is frequently associated with an increase in bacterial counts and the risk of infections among diabetic patients.[2] Changes in salivary composition have been suggested to affect the development, symptoms and severity of many oral diseases, particularly in diabetic patients.[23] These results were consistent with the results of the current study. An adequate level of salivary calcium, phosphate and fluoride is also involved in the significant deposition of these minerals in plaque, which greatly reduces the development of caries in the adjacent enamel of teeth.[12] Therefore, considering the incidence and characteristics of oral mucosal lesions among DM patients can be useful in planning, preventing and reducing the incidence of these lesions.[9]

To the best of our knowledge, as of today there is no study related to gender and salivary parameters. Consistent with our results, one study[24] reported no significant relationship between salivary glucose and gender. In addition, we found that calcium and total protein levels had a significant correlation with gender. Prospective studies with a larger sample size and different ethnicities are required to confirm our findings.

A number of studies have shown that salivary glucose[15,16,24] and HbA1c levels[24,25] have a significant correlation with blood glucose levels in DM patients, indicating that salivary glucose levels could be used as a monitoring tool to predict glycaemic control in diabetic patients. One study[25] and the present study did not show any significant correlation between salivary glucose levels and blood glucose levels. Also, the present study showed contradictory results for correlation between HbA1c levels and blood glucose levels in DM patients compared to other studies cited above. These contradictory results can be due to differences in the types of treatments, diabetes periods, age, gender, ethnicity and nutrition in DM patients. The simple and non-invasive collection of saliva samples is an important advantage that supports the usefulness of this fluid as a diagnostic tool compared with serum.

However, more studies are needed to be conducted in the future. Till that happens, taking the changes of these salivary parameters into account can be helpful for better therapeutic aims in DM patients as well as the possibility of reducing the risk of oral diseases in their follow-ups.

Conclusion

Salivary pH, urea, calcium, phosphorus, glucose and total protein were found to be important biochemical parameters for screening, diagnosis and monitoring of T2DM, and some parameters can be related to oral diseases in diabetic patients.

Disclaimer: None.

Conflict of Interest: None.

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