Original Article

Estimation and comparison of salivary flow rate and its composition in diabetic patients and nondiabetic patients: A pilot study

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Abstract

Background: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia irregularities in the metabolism of carbohydrates, lipids and protein. It is often associated with the development of microvascular and macrovascular complications and neuropathies. The health of oral tissues is known to be related to the quality and quantity of saliva both of which may be altered in diabetes.

Aim: The aim of the present study was to determine the salivary flow rate, electrolytes and total proteins in saliva of Type II diabetic patients.

Materials and Methods: A total number of 120 participants were included in this study, in which 80 patients were suffering from Type II DM (which included both controlled and uncontrolled diabetes) and 40 nondiabetic persons (controls). The study population included both the genders, with an age range of 40–70 years. The study population was divided into three groups.

Results: The values of total protein, sodium, potassium and salivary flow rate among controls, controlled diabetes and uncontrolled diabetes were collected, formulated and multiple comparisons between the groups using the analysis of variance and *post hoc* Tukey honestly significant difference analysis were done in version 16.0 of SPSS software.

Conclusion: Studies with larger sample size are warranted to know the exact pathophysiology of controlled and uncontrolled Type II DM in terms of salivary flow rate, salivary electrolytes and total protein.

Keywords: Diabetes mellitus, potassium, saliva, salivary flow rate, sodium, total protein

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia irregularities in the metabolism of carbohydrates, lipids and protein. It is often associated with the development of microvascular and macrovascular complications and neuropathies.[1] As the disease progresses, tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications

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and ulceration.[2,3] Thus, diabetes is known as a complex disease with deleterious effects on the general health of an individual.

Various studies have established diabetes as a risk factor for the development of oral diseases in humans.[4] It is probably the most common condition with salivary implication.

The health of oral tissues is known to be related to the quality and quantity of saliva both of which may be altered in diabetes. Several studies have been conducted to investigate salivary composition in participants with various systemic diseases.[5,6] Conditions such as dental caries and periodontitis have been long identified as the recognizable features of DM. Furthermore, majority of patients with diabetes, complain of xerostomia (dry mouth) due to overall decrease in flow of saliva due to systemic dehydration and an increase in the salivary glucose level.[7] Various underlying pathologies such as reduced salivary flow, delayed wound healing and atherosclerosis have been suggested to explain the increased prevalence of oral diseases in individuals with diabetes; however, the composition of saliva in these conditions needs further research.

Since diabetes is known to influence the salivary composition and function, the present study was carried out to estimate salivary flow rate, electrolytes and total proteins in Type 2 diabetes and to assess the correlation between the nondiabetic, controlled diabetic and uncontrolled diabetic patients using standard procedure. The aim of the present study was to determine the salivary flow rate, electrolytes and total proteins in saliva of Type II diabetic patients and use the results to follow-up and manage the diabetes for oral health issues.

MATERIALS AND METHODS

Patient selection

A total number of 120 participants were included in this study, in which 80 patients were suffering from Type II DM (which included both controlled and uncontrolled diabetes) and 40 nondiabetic persons (controls). Study population included both the genders, with an age range of 40–70 years.

The study population was divided into three groups.

Group I: (nondiabetes)

Group I comprised 40 patients 40–70 years of age with random nonfasting plasma glucose values ≥80 mg/dl and ≤120 mg/dl.

Group II: (controlled diabetes)

Group II comprised of 40 patients 40–70 years of age who were being treated for diabetes and had random nonfasting plasma glucose values >120 mg/dl and ≤200 mg/dl.

Group III: (uncontrolled diabetes)

Group III comprised of 40 patients 40–70 years of age who were being treated for diabetes and had random nonfasting plasma glucose values >200 mg/dl.

Inclusion criteria

- Patients having Type II diabetes
- Voluntary participation
- Sex: Both the genders.

Exclusion criteria

- Patients having other systemic diseases and on regular medication for the same
- Pregnant women
- Physically and mentally challenged persons.

Sample collection

All participants were explained in detail about this study and an informed consent was obtained in their native languages to prevent language bias and later was subjected to collection of saliva.

Saliva collection was undertaken between 10 and 11 a.m., and participants were instructed to have their breakfast not later than 8 a.m. Un-stimulated saliva was collected by the spitting method.

"Spit technique" was used for collection.[8] The patient was made to sit in the chair with head tilted forward. They were instructed not to speak, swallow or do any head movements during the procedure. The patient was instructed to spit in a sterile graduated container every minute for 10 min.

Salivary flow rate was calculated for every patient by using the formula

$$\text{Salivary Flow Rate} = \frac{\text{Post weight measure - Pre weight measure}}{\text{Collection period}}$$

= g/minute

Unstimulated saliva of 2 ml collected was used to evaluate electrolytes such as sodium, potassium and total proteins.

The testing of salivary samples was done in aseptic conditions. The unstimulated saliva of subjects was collected in a preweighed containers and immediately after collection, the bottles were examined to determine

the volume and stored at −200C until used for laboratory analysis. Samples were defrosted at the room temperature and then centrifuged at 6000 rpm for 10 min before being used to remove contaminants such as oral epithelial cells, micro-organisms and food debris among others.

The specimens were analyzed in the room temperature and were fed into automated analyzer for interpretation of the following parameters:

Salivary ions analysis

The saliva collected was analyzed for the concentrations of potassium (K+), sodium (Na+). For the determination of salivary ions, saliva was diluted at either 1/100 or 1/1000 and K+, Na + concentrations were determined using Roche 9180 electrolyte analyzer.

Salivary analysis of total protein

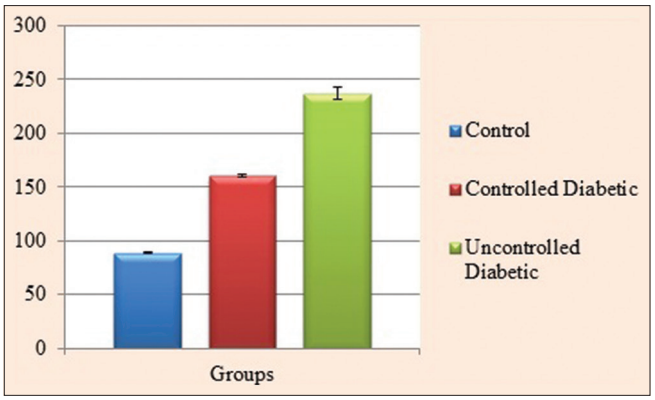
Saliva samples were defrosted at the room temperature and then centrifuged at 6000 rpm for 10 min before use. Total protein concentration expressed as mg/dl was determined using established automatic analyzer.

RESULTS

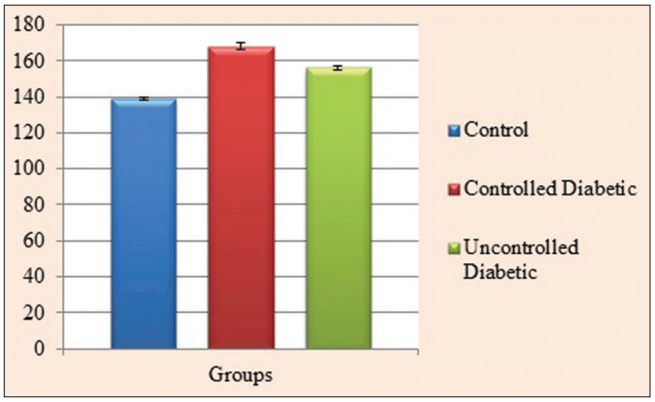
The values of total protein, sodium, potassium and salivary flow rate among controls, controlled diabetes and uncontrolled diabetes were collected, formulated and multiple comparisons between groups using analysis of variance and *post hoc* Tukey honestly significant difference analysis were done in Version 16.0 Statistical Package for the Social Sciences (SPSS), IBM Corporation, Chicago, United States of America.

The values of fasting blood sugar level in the Group 1 were in the range from 79 mg/dL to 96 mg/dL with an average of 88.9 mg/dL [Table 1 and Graph 1]. The values of sodium of the Group 1 in the range from 132 mEqL to 149 mEqL with an average of 139.05 mEqL [Table 1 and Graph 2]. The values of potassium of the Group 1 were in the range from 3.4 mEqL to 4.9 mEqL with an average of 4.04 mEqL [Table 1 and Graph 3]. The values of total protein of the Group 1 were in the range from 6.0 g/dL to 9.2 g/dL with an average of 7.28 g/dL [Table 1 and Graph 4]. The values of salivary flow rate in the Group 1 were in the range from 0.6 ml/min to 1.6 ml/min with an average of 1.09 ml/min [Table 1 and Graph 5].

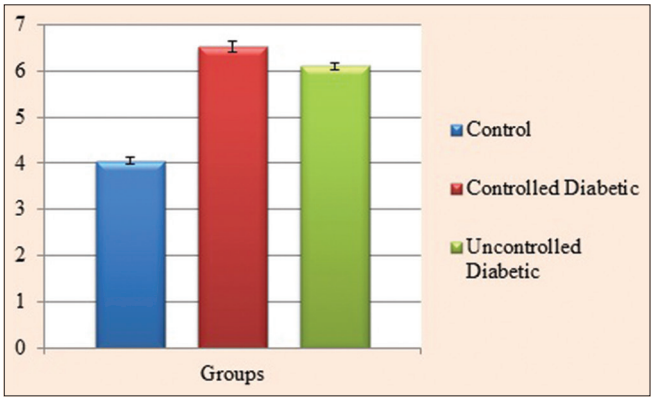
The values of fasting blood sugar level in the Group 2 were in the range from 142 mg/dL to 178 mg/dL with an average of 160.35 mg/dL [Table 1 and Graph 1]. The values of sodium of the Group 2 in the range from 146 mEqL to 185 mEqL with an average of 168.15 mEqL [Table 1 and Graph 2]. The values of potassium of the Group 2 were in the range from 8.5 mEqL to 10.6 mEqL with an average of 9.45 mEqL [Table 1 and Graph 3]. The values of total protein of the Group 2 were in the range from 5.2 g/dL to 8.2 g/dL with an average of 6.53 g/dL [Table 1 and Graph 4]. The values of salivary flow rate in the Group 2 were in the range from 0.4 ml/min to 1.0 ml/min with an average of 0.63 ml/min [Table 1 and Graph 5].



Graph 1: Comparison of total blood sugar level between case and control group



Graph 2: Comparison of sodium level between case and control group

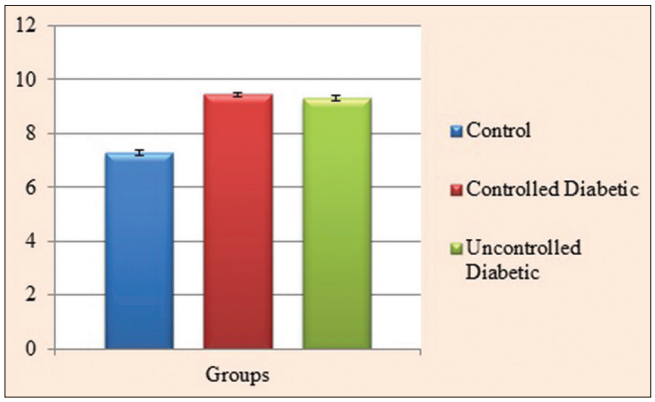


Graph 3: Comparison of potassium level between case and control group

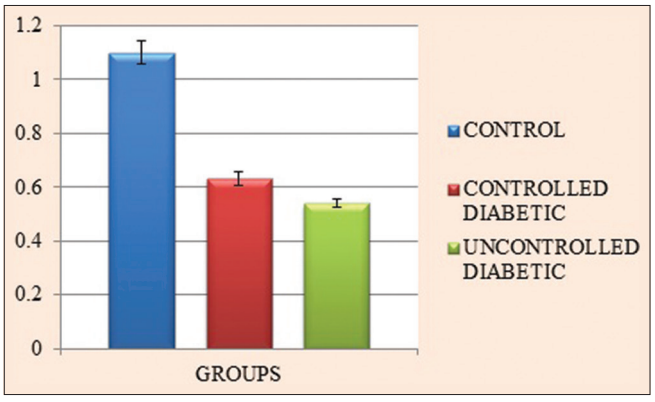
Table 1: Quantitative data of fasting blood sugar, sodium, potassium, total protein levels and salivary flow rate between case and control group

	n	Mean	SD	SE	95% CI for mean		Minimum	Maximum
					Lower bound	Upper bound		
Age								
Control	40	38.8750	8.71247	1.37756	36.0886	41.6614	27.00	66.00
Controlled diabetic	40	51.0000	11.71018	1.85154	47.2549	54.7451	29.00	82.00
Uncontrolled diabetic	40	56.1250	8.97343	1.41882	53.2552	58.9948	44.00	78.00
Total	120	48.6667	12.20506	1.11416	46.4605	50.8728	27.00	82.00
Blood sugar								
Control	40	88.9000	4.41907	0.69872	87.4867	90.3133	79.00	98.00
Controlled diabetic	40	1.6035E2	10.06785	1.59187	157.1301	163.5699	142.00	178.00
Uncontrolled diabetic	40	2.3702E2	35.31723	5.58415	225.7300	248.3200	186.00	308.00
Total	120	1.6209E2	64.32337	5.87189	150.4647	173.7186	79.00	308.00
Sodium								
Control	40	1.3905E2	3.69997	0.58502	137.8667	140.2333	132.00	149.00
Controlled diabetic	40	1.6815E2	10.22704	1.61704	164.8792	171.4208	146.00	185.00
Uncontrolled diabetic	40	1.5630E2	7.71678	1.22013	153.8321	158.7679	144.00	178.00
Total	120	1.5450E2	14.22095	1.29819	151.9295	157.0705	132.00	185.00
Potassium								
Control	40	4.0450	0.47283	0.07476	3.8938	4.1962	3.40	5.10
Controlled diabetic	40	6.5325	0.69629	0.11009	6.3098	6.7552	5.20	8.20
Uncontrolled diabetic	40	6.0575	0.40439	0.06394	5.9282	6.1868	5.30	6.90
Total	120	5.5450	1.20753	0.11023	5.3267	5.7633	3.40	8.20
Total protein								
Control	40	7.2875	0.73909	0.11686	7.0511	7.5239	6.00	9.20
Controlled diabetic	40	9.4525	0.51340	0.08118	9.2883	9.6167	8.50	10.60
Uncontrolled diabetic	40	9.3025	0.57490	0.09090	9.1186	9.4864	8.50	10.70
Total	120	8.6808	1.16463	0.10632	8.4703	8.8913	6.00	10.70
Salivary flow rate								
Control	40	1.0975	0.26553	0.04198	1.0126	1.1824	0.60	1.60
Controlled Diabetic	40	0.6300	0.16361	0.02587	0.5777	0.6823	0.40	1.10
Uncontrolled Diabetic	40	0.5400	0.09001	0.01423	0.5112	0.5688	0.40	0.70
Total	120	0.7558	0.30782	0.02810	0.7002	0.8115	0.40	1.60

SD: Standard deviation, SE: Standard error, CI: Confidence interval



Graph 4: Comparison of protein level between case and control group



Graph 5: Comparison of salivary flow rate between case and control group

The values of fasting blood sugar level in Group 3 were in the range from 186 mg/dL to 303 mg/dL with an average of 237 mg/dL [Table 1 and Graph 1]. The values of sodium of the Group 3 were in the range from 144 mEqL to 178 mEqL with an average of 156.3 mEqL [Table 1 and Graph 2]. The values of potassium of the Group 3 were in the range from 5.3 mEql to 6.9 mEqL with an average of 6.05 mEqL [Table 1 and Graph 3]. The values of total protein of Group 3 were in the range from 8.5 g/dl to 10.7 g/dl with an average of 9.3025 g/dl [Table 1 and Graph 4]. The values of salivary flow rate in the Group 3 were in the range from 0.4 ml/min to 0.7 ml/min with an average of 0.54 ml/min [Table 1 and Graph 5].

There was a distinct increase in values of total protein, sodium, potassium and decrease in salivary flow rate among the controlled diabetic and uncontrolled diabetic group. The values were found to be statistically significant ($P < 0.05$) [Table 2]. Within the groups of controlled and uncontrolled diabetes, there seemed to be

Table 2: Comparative analysis of total protein, sodium, potassium levels and salivary flow rate between controlled and uncontrolled diabetes mellitus group

Parameter	Groups (mean±SD)			*P*
	Control	Controlled diabetic	Uncontrolled diabetic	
Age	52.68±9.27	51.0±11.71	56.13±8.97	0.072
Blood sugar	88.9±4.42	160.35±10.07	237.02±35.32	0.0001
Sodium	139.05±3.70	168.15±10.23	156.3±7.71	0.0001
Potassium	4.05±0.47	6.53±0.70	6.1±0.40	0.0001
Total protein	7.29±0.74	9.45±0.51	9.30±0.57	0.0001
Salivary flow rate	1.10±0.27	0.63±0.16	0.54±0.09	0.0001

SD: Standard deviation

Table 3: ANOVA analysis between and within groups

	Sum of squares	df	Mean square	*F*	Significance
Age					
Between groups	6277.917	2	3138.958	32.078	0.000
Within groups	11448.750	117	97.853		
Total	17726.667	119			
Blood sugar					
Between groups	439002.317	2	219501.158	481.293	0.000
Within groups	53359.675	117	456.066		
Total	492361.992	119			
Sodium					
Between groups	17130.600	2	8565.300	144.496	0.000
Within groups	6935.400	117	59.277		
Total	24066.000	119			
Potassium					
Between groups	139.513	2	69.756	240.012	0.000
Within groups	34.004	117	0.291		
Total	173.517	119			
Total protein					
Between groups	116.933	2	58.466	153.813	0.000
Within groups	44.473	117	0.380		
Total	161.406	119			
Salivary flow rate					
Between groups	7.166	2	3.583	102.006	0.000
Within groups	4.110	117	0.035		
Total	11.276	119			

an increase in the values of controlled diabetic group than the uncontrolled group which also showed a statistical significance [Table 3]. However, total protein and salivary flow rate was not statistically significant, even though the values in the controlled diabetic group were higher than that of uncontrolled group [Table 4].

DISCUSSION

DM is a common metabolic disease affecting the salivary gland functioning and thus altering the salivary constituents.[9] Murrah *et al*.[10] have proved that changes in basement membrane of the parotid gland could alter the ability of the glands to transfer molecules, electrolytes and water resulting in altered salivary output.

The aim of this study was to estimate the salivary flow rate, electrolytes and total protein in the diabetic patients and also to compare them between the controlled and uncontrolled diabetic patients. The study population (n = 120) was divided into three groups, namely Group 1, i.e., healthy subject (n = 40), Group 2, i.e., controlled diabetes (n = 40) and Group 3, i.e., uncontrolled diabetes (n = 40). Saliva was collected from the sample population and was biochemically analyzed.

In this present study, the total protein level is increased in the diabetic patients (Group 3) when compared to nondiabetic participants. This is in agreement with Arati *et al*.[11] and Streckfus *et al*.[12] who demonstrated highly significant positive correlations in salivary total protein levels among uncontrolled and controlled diabetic groups. This could be attributed to the increase in basement membrane permeability, allowing easy and increased passage of serum proteins into the whole saliva through salivary gland and gingival crevices.

Mata *et al*.[13] reported increased salivary protein concentration in diabetic patients, which was attributed to reduced salivary fluid secretion. This study is also in agreement with our findings where salivary flow rate is inversely proportional to the total protein level [Table 4, Graphs 4 and 5].

In this current study, we found statistically significant differences in salivary flow rate between controlled, uncontrolled diabetic group and healthy non diabetic group [Table 4 and Graph 5]. Salivary flow rate is decreased in diabetes patients when compared to the healthy participants [Graph 5].

The decrease in salivary flow rate occurring in diabetes can be factorial, either due to fatty infiltration of cells into the salivary glands or physical alteration of mucosal cells subsequent to dehydration due to polyuria or microvascular disease. It can also be due to local inflammation and irritation in the oral cavity, metabolic disturbances and neuropathy affecting the salivary glands or as a result of drug therapy for diabetes and concomitant drugs.

The result of the study done by Meurman *et al*.[14] contradicted with the finding of the present study as it showed no significant differences in the salivary flow rate. This may be attributed to the differences in sample selection and variation of environmental factors.

With respect to potassium, salivary concentration of this ion was found to be increased in diabetic patients when compared with nondiabetic individuals in the present study. Similar finding had been reported by Lasisi and Fasanmade,[15] Mata *et al*.[13]

Table 4: Multiple comparisons between the case group (controlled and uncontrolled diabetes mellitus) and control group

Dependent variable	Groups (I)	Groups (J)	Mean difference (I-J)	SE	Significant	95% CI	
						Lower bound	Upper bound
Age	Control	Controlled diabetic	−12.12500*	2.21193	0.000	−17.3759	−6.8741
		Uncontrolled diabetic	−17.25000*	2.21193	0.000	−22.5009	−11.9991
	Controlled diabetic	Control	12.12500*	2.21193	0.000	6.8741	17.3759
		Uncontrolled diabetic	−5.12500	2.21193	0.057	−10.3759	0.1259
	Uncontrolled diabetic	Control	17.25000*	2.21193	0.000	11.9991	22.5009
		Controlled diabetic	5.12500	2.21193	0.057	−0.1259	10.3759
Blood sugar	Control	Controlled diabetic	−71.45000*	4.77528	0.000	−82.7861	−60.1139
		Uncontrolled diabetic	−148.12500*	4.77528	0.000	−159.4611	−136.7889
	Controlled diabetic	Control	71.45000*	4.77528	0.000	60.1139	82.7861
		Uncontrolled diabetic	−76.67500*	4.77528	0.000	−88.0111	−65.3389
	Uncontrolled diabetic	Control	148.12500*	4.77528	0.000	136.7889	159.4611
		Controlled diabetic	76.67500*	4.77528	0.000	65.3389	88.0111
Sodium	Control	Controlled diabetic	−29.10000*	1.72158	0.000	−33.1869	−25.0131
		Uncontrolled diabetic	−17.25000*	1.72158	0.000	−21.3369	−13.1631
	Controlled diabetic	Control	29.10000*	1.72158	0.000	25.0131	33.1869
		Uncontrolled diabetic	11.85000*	1.72158	0.000	7.7631	15.9369
	Uncontrolled diabetic	Control	17.25000*	1.72158	0.000	13.1631	21.3369
		Controlled diabetic	−11.85000*	1.72158	0.000	−15.9369	−7.7631
Potassium	Control	Controlled diabetic	−2.48750*	0.12055	0.000	−2.7737	−2.2013
		Uncontrolled diabetic	−2.01250*	0.12055	0.000	−2.2987	−1.7263
	Controlled diabetic	Control	2.48750*	0.12055	0.000	2.2013	2.7737
		Uncontrolled diabetic	0.47500*	0.12055	0.000	0.1888	0.7612
	Uncontrolled diabetic	Control	2.01250*	0.12055	0.000	1.7263	2.2987
		Controlled diabetic	−0.47500*	0.12055	0.000	−0.7612	−0.1888
Total protein	Control	Controlled diabetic	−2.16500*	0.13786	0.000	−2.4923	−1.8377
		Uncontrolled diabetic	−2.01500*	0.13786	0.000	−2.3423	−1.6877
	Controlled diabetic	Control	2.16500*	0.13786	0.000	1.8377	2.4923
		Uncontrolled diabetic	0.15000	0.13786	0.523	−0.1773	0.4773
	Uncontrolled diabetic	Control	2.01500*	0.13786	0.000	1.6877	2.3423
		Controlled diabetic	−0.15000	0.13786	0.523	−0.4773	0.1773
Salivary flow rate	Control	Controlled diabetic	0.46750*	0.04191	0.000	0.3680	0.5670
		Uncontrolled diabetic	0.55750*	0.04191	0.000	0.4580	0.6570
	Controlled diabetic	Control	−0.46750*	0.04191	0.000	−0.5670	−0.3680
		Uncontrolled diabetic	0.09000	0.04191	0.085	−0.0095	0.1895
	Uncontrolled diabetic	Control	−0.55750*	0.04191	0.000	−0.6570	−0.4580
		Controlled diabetic	−0.09000	0.04191	0.085	−0.1895	0.0095

*The mean difference is significant at the 0.05 level. CI: Confidence interval, SE: Standard error

Study done by Ben-Aryeh *et al*.[16] Iis also in accordance with our findings. Elevation of potassium concentration in saliva of diabetic patients is probably secondary to diabetes induced decrease in salivary fluid output.[13] This might be due to intact secretory capacity of the salivary glands in Type 2 diabetes. In contrast, Streckfus *et al*.[12] and Marder *et al*.[17] documented that there is no difference in the potassium level in diabetic patients in their studies.

The salivary concentration of sodium was found to be increased in the diabetes group when compared to the controlled group the present study. This finding is in positive agreement with the study conducted by Basavaraj *et al*.[18] The reason could be due to decreased salivary flow rate which in turn increases the concentration of the sodium ion in saliva of diabetic patients.

In contrast, a study done by Lasisi and Fasanmade[15] found no significant difference in salivary sodium level in their diabetic patient's sample.

On intergroup comparison in the present study, barring salivary flow rate and total protein level, electrolytes such as sodium and potassium showed statistically significant increase in controlled diabetics against the uncontrolled ones. This can be attributed to the following probable reasons:

- Smaller sample size
- Compromise of salivary flow in poorly controlled diabetes. This is in accordance with the study done by Rosamund and William[19] which in turn leads to altered salivary flow rate
- Effect of certain drugs taken by the study group volunteers for other underlying systemic diseases, which may not have been disclosed by them.

CONCLUSION

Thus, studies with larger sample size are warranted to know the exact pathophysiology of controlled and uncontrolled Type II DM in terms of salivary flow rate, salivary electrolytes and total protein.

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Conflicts of interest

There are no conflicts of interest.

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