

Correlation between Salivary Glucose and Blood Glucose and the Implications of Salivary Factors on the Oral Health Status in Type 2 Diabetes Mellitus Patients

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INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome characterized by hyperglycemia because of absolute or relative deficiency of insulin.^[1] The diagnosis of DM is based on blood glucose estimations. Blood collection is an invasive procedure, and may be traumatizing, especially in diabetic patients who require routine daily monitoring of blood glucose levels. Ongoing research in the past few decades has focussed on alternative methodologies that involve incorporating various other body fluids that could be used as a substitute for blood for diagnostic purposes. One of the most important among these is saliva. Alterations in the salivary flow and composition of saliva in diabetics have been reported in numerous previous studies, although the findings have frequently been contradictory. There still is no consensus about which parameters should be followed in saliva of type 2 DM patients to enable a salivary diagnosis of type 2 DM.^[2-4]

MATERIALS AND METHODS

Ethical clearance for the study was obtained from the Institutional Review Board. Participants were informed about

ABSTRACT

Aims and Objectives: The purpose of this study was to estimate and assess any correlation between random capillary blood glucose (RCBG) and unstimulated whole salivary glucose (UWSG), as well as to estimate various salivary parameters, such as flow rate, pH, buffering capacity, and the influence of these factors on the oral health status in type 2 diabetes mellitus (DM).

Materials and Methods: Sixty individuals suffering from type 2 DM and 40 healthy individuals in the age group of 30–60 years were included in the study. RCBG was estimated using glucometer and UWSG was estimated using photocolormeter. Salivary parameters such as flow rate, pH, and buffering capacity were assessed using GC[®] Saliva kit. Oral health status was recorded using the Russell's periodontal index (RPI) and the Decayed Missing Filled Teeth (DMFT) index. The Statistical Package for the Social Sciences version 16 was used for statistical analysis.

Results: Type 2 diabetics had higher mean values for RCBG levels and UWSG. Type 2 diabetics had low mean salivary flow rate, pH, and buffering capacity. Type 2 diabetics had higher mean values for RPI.

Conclusion: Among the salivary factors studied, salivary glucose significantly influenced the periodontal status in Type 2 diabetics.

KEYWORDS: Buffering capacity, flow rate, pH, Type 2 Diabetes mellitus, unstimulated whole salivary glucose

the study protocol, and only those who provided their written consent were included in the study. This cross-sectional study was conducted over a period of 8 months from June 2010 to February 2011. Based on the available literature of cross-sectional, observational studies which included sample sizes that ranged 40–180, in this study the sample size was considered to be 100, which included 60 diabetics and 40 healthy controls.^[5-11]

INCLUSION CRITERIA

Sixty patients previously diagnosed with type 2 DM and with no other systemic illness, and 40 healthy volunteers with no apparent medical history in the age group of 30–60 years were randomly selected and included in the study.

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EXCLUSION CRITERIA

- Completely edentulous patients
- Patients with any oral mucosal lesions
- Patients on any medications other than for type 2 DM
- Tobacco/betel chewing habits.

Random capillary blood glucose (RCBG) was estimated using sterile lancets and SD check Gold® Glucometer with glucose reagent strips using the finger prick method. Saliva samples were collected in the morning between 9 AM and 12 PM. Before collecting the saliva samples, patients were asked to rinse their mouth with 200 ml water. Patients were seated in an upright position during saliva sample collection. They were asked to spit into the graduated disposable collecting cup at the end of every minute for 5 minutes and the average was calculated to estimate the Unstimulated whole salivary flow rate. Salivary pH was estimated by placing the GC® saliva pH strip for 10 seconds in the saliva sample, which was then removed and matched with the color-coded table provided along with the kit. Salivary buffering capacity was estimated using the GC® Saliva buffer strips. Pipettes provided along with the kit were used to draw saliva sample from the collecting cup and 3 drops added over the 3 slots of the strips. After 2 minutes, the color change was matched with the color-coded table scores provided by the manufacturer. Periodontal status was assessed according to the Russell's periodontal index (RPI). Decayed Missing Filled Teeth (DMFT) index was recorded to assess the status of teeth.

Using a micropipette, 10 µl of saliva was drawn from the disposable collecting cup and added into a cuvette to which 1000 µl of glucose oxidase–peroxidase enzyme reagent was added; the sample was then incubated at 37°C for 10 minutes. Similarly, 10 µl of standard glucose solution was drawn into a cuvette to which 1000 µl of enzyme reagent was added, and the sample was incubated at 37°C for 10 minutes. The optical absorbance readings were recorded using the Digital photocolormeter using the green filter with a peak of 540 nm wavelength [Figure 1].



Figure 1: Armamentarium used to assess unstimulated whole salivary glucose

UWSG was calculated using the formula:

Salivary glucose in mg/dl = Absorbance of sample × Concentration of standard/absorbance of standard

Concentration of the standard glucose was 100 mg/dl

Statistical analysis was done using contingency coefficient analysis, independent samples *t*-test, multivariate analysis of variance (MANOVA), and correlations using Pearson coefficient. The Statistical Package for the Social Sciences (SPSS) for Windows (SPSS, version 16.0, Chicago, SPSS Inc.) was used for statistical analysis.

RESULTS

The mean RCBG and UWSG levels in type 2 diabetics were 180 mg/dl and 12.9 mg/dl, respectively [Table 1]. The mean RCBG and UWSG levels in healthy controls were 95.1 mg/dl and 9.46 mg/dl, respectively [Table 1]. The difference between the groups was statistically significant ($P = 0.000$) [Table 2].

A positive correlation between RCBG and UWSG was observed in both the study and control groups [Graphs 1 and 2]. The mean unstimulated whole salivary flow rate in type 2 diabetics was 0.6 ml/min and in the healthy controls it was 0.67 ml/min [Table 1]. The difference in unstimulated whole salivary flow rate between the groups was statistically significant ($P = 0.029$) [Table 2]. The mean unstimulated salivary pH in type 2 diabetics was 6.8 and in the healthy controls it was 7.1 [Table 1]. The difference between the groups was significant statistically ($P = 0.007$).

The mean salivary buffering capacity in type 2 diabetics was 7 and in the healthy controls it was 8.4 [Table 1]. There was a statistically significant difference in the salivary buffering capacity between the groups ($P = 0.021$) [Table 2].

No significant correlation between salivary flow rate and the salivary buffering capacity was observed in type 2 diabetics, however, a statistically significant correlation ($P = 0.000$) was found between salivary flow rate and salivary pH [Table 3].

The values of salivary pH and salivary buffering capacity showed good correlation in both the type 2 diabetics and the control group, as the salivary pH decreased the salivary buffering capacity also decreased and the relationship was highly significant statistically ($P = 0.000$) [Table 2].

The mean values for RPI in the type 2 diabetics was found to be 2.1, and in the healthy controls it was 1.2 [Table 1]. The difference in the RPI scores between the groups was significant statistically ($P = 0.000$) [Table 2].

The mean DMFT scores in type 2 diabetics was 5.7 and in the healthy controls it was 5.8 [Table 1]. The difference in the DMFT scores between the groups was not significant statistically.

The RPI scores of type 2 diabetics showed positive correlation with only the salivary glucose levels among the various tested parameters. None of the tested salivary factors showed any statistically significant effect on the RPI scores in the control group.

Table 1: Descriptive statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Blood Glucose	Control	40	95.1000	15.09423	2.38661
	Type 2 DM	60	180.9833	79.66976	10.28532
Salivary pH	Control	40	7.0600	0.39794	0.06292
	Type 2 DM	60	6.8333	0.41197	0.05318
Salivary Buffering Capacity	Control	40	8.4250	2.62031	0.41431
	Type 2 DM	60	7.1667	2.64362	0.34129
Salivary Flow Rate	Control	40	0.6650	0.14242	0.02252
	Type 2 DM	60	0.6000	0.14380	0.01856
Russell's Periodontal Index	Control	40	1.2275	0.56067	0.08865
	Type 2 DM	60	2.0985	0.53975	0.06968
DMFT	Control	40	5.8250	4.10683	0.64935
	Type 2 DM	60	5.7500	3.53493	0.45636
Salivary Glucose	Control	40	9.4615	1.64339	0.25984
	Type 2 DM	60	12.9075	3.38226	0.43665

Table 2: Independent samples test: Control and type 2 DM*t*-test for equality of means

		t	df	Sig. (two-tailed)	Mean Difference
Blood Glucose	Equal variances assumed	-6.727	98	0.000	-85.8833
Salivary pH	Equal variances assumed	2.732	98	0.007	0.2267
Salivary Buffering Capacity	Equal variances assumed	2.340	98	0.021	1.2583
Salivary Flow Rate	Equal variances assumed	2.223	98	0.029	0.0650
Russell's Periodontal Index	Equal variances assumed	-7.784	98	0.000	- .8710
DMFT	Equal variances assumed	0.097	98	0.923	0.0750
Salivary Glucose	Equal variances assumed	-5.983	98	0.000	-3.4460

df, degree of freedom

The caries experience of both type 2 diabetics and controls in our study was similar with no statistical difference in the DMFT scores [Table 3].

DISCUSSION

Epidemiological studies in India have shown high prevalence of type 2 DM; in the year 2002, it was estimated that there were 19.4 million individuals affected by type 2 DM, which is likely to increase up to 57.2 million by the year 2025.^[3] Routine blood examination for glucose assessment can be traumatizing to the patient, and hence, other alternatives have been explored, among which salivary diagnostics hold much promise. Saliva-based diagnostics are not limited to oral diseases but have been extended to the entire physiologic system, as most compounds found in the blood are also present in the saliva. Accordingly, saliva can reflect the physiologic state of the body including emotional, endocrinal, nutritional, and metabolic variations, and acts as a source for monitoring oral and systemic health.^[4]

A systematic review of previously published studies reflects the fact that salivary glucose concentration increases in type 2 DM, and a positive correlation exists between blood glucose and salivary glucose; hence, it can be a useful biomarker to monitor type 2 DM.^[12] In the present study, UWSG was found to reflect the RCBG levels in both the groups. Type 2 diabetics had significantly higher USWG/RCBG levels than the controls,

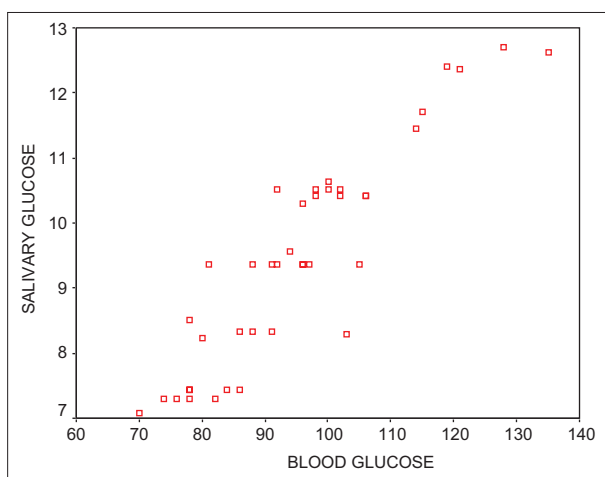
a fact which has been documented in previous studies.^[5,13-19] The correlation between RCBG and UWSG could plausibly be because of leakage of glucose from blood across the basement membrane of salivary glands. Microvascular alterations in the blood vessels that are commonly seen in type 2 diabetics could also contribute to increased salivary glucose levels.^[20,21] Saliva samples collected in the present study represented the whole mouth fluid, and therefore, reflects glucose levels not only due to leakage across the basement membrane of major and minor salivary glands but also from the gingival crevicular fluid. Furthermore, it has been proposed by Belazi^[22] that the basement membrane alterations lead to enhanced leakage of serum components including glucose into the gingival crevicular fluid rather than into saliva. However, in contrast to the present study, various other authors^[13,23,24] could not establish any correlation between RCBG and UWSG.

The decrease in the unstimulated whole salivary flow rate in type 2 diabetics is in accordance with previous studies.^[8,10,13,16,25-27] Type 2 DM is known to affect the sympathetic and parasympathetic nervous system of the salivary glands, resulting in decreased salivary secretion, microangiopathy, dehydration, and hormonal changes, which may contribute to the decrease in the salivary flow rate.^[13] However, few authors^[28,29] were not able to establish significant difference in salivary flow rates between type 2 DM and healthy controls. We found a significant difference

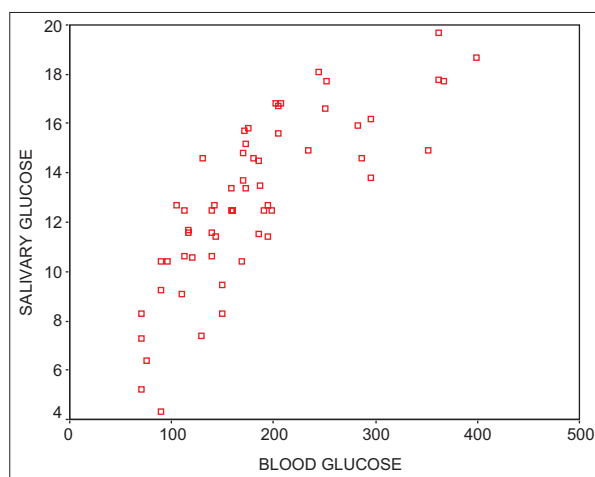
Table 3: Correlations in the study group (Type 2 diabetics and healthy volunteers) among various parameters

		Blood Glucose	DMFT	Russell's Periodontal Index	Salivary pH	Salivary Buffering Capacity	Salivary Glucose	Salivary Flow Rate
Blood Glucose	Pearson Correlation	1	0.032	0.576(**)	-0.193	-0.233(*)	0.840(**)	-0.088
	Sig. (two-tailed)		0.749	0.000	0.054	0.020	0.000	0.384
	N	100	100	100	100	100	100	100
DMFT	Pearson Correlation	0.032	1	-0.015	-0.038	-0.172	0.060	0.034
	Sig. (two-tailed)	0.749		0.882	0.710	0.087	0.555	0.733
	N	100	100	100	100	100	100	100
Russell's Periodontal Index	Pearson Correlation	0.576(**)	-0.015	1	-0.296(**)	-0.207(*)	0.522(**)	-0.206(*)
	Sig. (two-tailed)	0.000	0.882		0.003	0.038	0.000	0.040
	N	100	100	100	100	100	100	100
Salivary pH	Pearson Correlation	-0.193	-0.038	-0.296(**)	1	0.582(**)	-0.180	0.382(**)
	Sig. (two-tailed)	0.054	0.710	0.003		0.000	0.073	0.000
	N	100	100	100	100	100	100	100
Salivary Buffering Capacity	Pearson Correlation	-0.233(*)	-0.172	-0.207(*)	0.582(**)	1	-0.248(*)	0.302(**)
	Sig. (two-tailed)	0.020	0.087	0.038	0.000		0.013	0.002
	N	100	100	100	100	100	100	100
Salivary Glucose	Pearson Correlation	0.840(**)	0.060	0.522(**)	-0.180	-0.248(*)	1	-0.065
	Sig. (two-tailed)	0.000	0.555	0.000	0.073	0.013	0.	0.519
	N	100	100	100	100	100	100	100
Salivary Flow Rate	Pearson Correlation	-0.088	0.034	-0.206(*)	0.382(**)	0.302(**)	-0.065	1
	Sig. (two-tailed)	0.384	0.733	0.040	0.000	0.002	0.519	
	N	100	100	100	100	100	100	100

**Correlation is significant at the 0.01 level (two-tailed); *Correlation is significant at the 0.05 level (two-tailed)



Graph 1: Scatter plot No.1 correlation between random capillary blood glucose and unstimulated whole salivary glucose in control group



Graph 2: Scatter plot No.2: Correlation between random capillary blood glucose and unstimulated whole salivary glucose in experimental group

in the salivary pH between the type 2 DM patients and control ($P < 0.01$), which was similar to other studies.^[16,20,27]

In accordance with previous studies,^[28,30] we found significant differences ($P < 0.05$) in the buffering capacity between type 2 DM and control groups. This can also be attributed to the hormonal and metabolic changes in diabetic patients causing

altered levels of salivary buffering systems. Results contrary to our study have been reported by Collin *et al.*^[31]

In the present study, there was significant correlation ($P < 0.01$) between the salivary flow rate and salivary pH in the diabetics, and such correlation was not observed in the control group individuals. Even though type 2 DM patients had significant

decrease in salivary flow rate in our study, it was observed that salivary flow rates were not as low as those in patients suffering from hyposalivation. This could be caused by increased fluid intake by diabetics due to polydipsia. Because buffering capacity is dependent on the pH levels, type 2 DM had salivary buffering capacity correlating with the salivary pH. Interestingly, it was observed that, in the control group, salivary pH levels were within the normal limits independent of the salivary flow rate. This could be due to reduced acidogenic flora in the oral cavity and increased salivary clearance activity maintaining normal pH levels.

Demmer *et al.* reported that, in patients with type 2 DM, the risk of periodontal disease is three times higher than that in the general population.^[32] Similarly, we found that the type 2 DM patients had significantly poor periodontal status than the healthy controls. This is in accordance to previous studies.^[7,33-36] It has been shown that DM causes alterations in the connective tissue metabolism by uncoupling the resorptive and formative processes, thus leading to increased levels of loss of periodontal attachment and bone loss.^[37]

Among all the parameters tested in our study, only RCBG and UWSG showed significant positive correlation ($P < 0.01$) with the RPI scores in type 2 DM patients. None of the other salivary parameters studied correlated with the RPI scores, indicating that the level of glycemic control is an important determinant in being a risk factor for the development of gingivitis and periodontitis in type 2 DM.

Studies concerning the occurrence of caries in diabetic patients have yielded controversial results. In the present study, no significant difference was observed in the DMFT scores between the type 2 DM and controls, similar to earlier studies.^[31,38] Lack of significant difference in the DMFT scores between the groups could be due to modification in the diet with reduced amounts of refined carbohydrate intake by the type 2 DM patients, thereby reducing the formation of an acidogenic environment. The fact that most of the patients who formed the study group belonged to the urban population and had unproblematic access to dental care could have also contributed to no significant differences in the mean DMFT scores between the type 2 DM and control groups. Our results are contrary to a few authors^[6,16,25,34,39,40] who have reported that diabetics have slightly higher mean DMFT scores than the controls.

CONCLUSION

From our results, it can be concluded that the salivary glucose levels reflect the random blood glucose levels. Type 2 diabetics have significantly lower salivary flow rate, pH, and buffering capacity and present with advanced periodontal destruction than the healthy population. Limitation of our study would be the relatively smaller sample size. Further studies with larger sample size are warranted to substantiate the correlation between blood glucose and salivary glucose to devise saliva-based tests for diagnosing DM. Fasting salivary glucose estimation would also be an interesting area for further research.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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