Determination of normal range for fasting salivary glucose in Type 1 diabetics

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Abstract

Background: The most commonly employed investigative procedure for monitoring glucose levels is blood investigation, which is invasive and gives discomfort to the patient. The purpose of the study was to validate a noninvasive, easy, and reliable method for predicting glucose levels in Type 1 diabetics and to validate a regression equation for converting the known values of salivary glucose to blood glucose. **Materials and Methods:** 200 volunteers consisting of 100 Type 1 diabetics and 100 healthy controls were included, and their fasting blood and salivary glucose levels were assessed, using a semi-auto analyzer. **Results:** On analysis of the data, statistically significant positive results were obtained ($P < 0.05$) when the blood and salivary glucose levels were considered among the study group participants, control group participants, and both study and control group participants. A cut-off value for salivary glucose (11.60 mg%) was defined, above which a person may be considered as diabetic. Also, the regression equation was obtained which could be used for the conversion of known value of salivary glucose to blood glucose and vice versa. **Conclusion:** The present study successfully demonstrated the role of saliva as a noninvasive and reliable marker for the prediction of glucose levels in Type 1 diabetics who show elevated blood glucose levels.

Key words: *Blood glucose, diabetes mellitus, monitoring therapy, noninvasive, salivary glucose*

INTRODUCTION

The first complete clinical description of diabetes appears to have been made by Aulus Cornelius Celsus (30 BC–50 AD).[1]

According to the American Diabetes Association, diabetes mellitus has been classified on the basis of etiology into Type 1 and Type 2, other specific types (which include genetic defects in β-cells, genetic defect in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug or chemical induced, infections, uncommon form of immune-mediated diabetes, other genetic syndromes sometimes associated with diabetes), and gestational diabetes mellitus.[2]

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Type 1 diabetes accounts for 5–10% of all cases. It results from a progressive, cellular-mediated autoimmune destruction of the pancreatic β-cells that leads to complete insulin deficiency.[3] Patients with Type 1 diabetes are severely insulin deficient and are dependent on insulin treatment for their survival.[4]

In 2000, the estimated global prevalence of diabetes among adults was 2.8%, or 171 million people. The prevalence of diabetes is expected to increase to 4.4%, or 366 million people, by 2030 globally.[5] In India, it is estimated that presently 19.4 million individuals are affected with this deadly disease, and the number is likely to go up to 57.2 million by the year 2056.[6]

The most commonly employed investigative procedure to diagnose diabetes mellitus is blood investigation which is invasive and offer discomfort to patients. Efforts are on, to derive methods which are noninvasive and cause less discomfort to patients. Saliva offers distinctive advantage as it can be collected noninvasively and with limited training, involves fewer complications, and is cost effective.[7] It is beautifully said by Mandel that 'saliva lacks the drama of blood, sincerity of sweat and emotional appearance of tears'.[8] Saliva acts as a mirror of the body and, hence, is a perfect medium to be explored for disease and health surveillance. Considering the ease, painless procedure,[9] and noninvasiveness of collecting salivary samples, and also the limited amount of studies that evaluated the concentration of glucose in saliva, this study was undertaken to authenticate the reliability of saliva as a biomarker for the prediction of glucose levels in Type 1 diabetes.

MATERIALS AND METHODS

Study design

A total of 200 subjects from the OPD of Department of Oral Medicine and Radiology were included in the study, after obtaining prior approval from the ethical committee of the college [Table 1]. Of these, 100 subjects formed the study group who had a positive history of Type 1 diabetes and were on insulin, but were free from any other systemic illness, were not on medications causing xerostomia, did not have any other obvious oral lesion, were not previously treated for any salivary gland disorder, and were not having any habit of tobacco chewing or smoking.

Glucose analysis

The analysis of glucose was done using GOD/POD (glucose oxidase-peroxidase) method, which is based on the principle that glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red-colored quinoneimine dye complex.

Sampling of blood

Fasting blood glucose sample was taken between 8am and 10am. Subjects were given prior instructions to remain on empty stomach. Two milliliters of blood was drawn from the median vein and was allowed to clot in the test tube. Afterward, it was centrifuged at 3000 rpm for 10 min and then the serum was separated. In another test tube, 10 μl of the serum was taken to which 1000 μl of the glucose reagent was added (provided by the manufacturer). This mixture was incubated (in-built in the semi-auto analyzer) for 10 min at 37°C.

Sampling of saliva

Immediately after collecting the blood glucose sample, the fasting saliva sample was taken. The subject was asked to rinse mouth thoroughly to remove any debris. Then he/she was instructed to keep his mouth open for 2–3 min and the saliva was allowed to pool on the floor of mouth. Unstimulated whole saliva was collected in a sterile container by asking the subject to expectorate the saliva into the container gradually over a period of 5–10 min till approximately 1 ml of saliva was collected. The salivary sample collected was then transferred to a test tube and subjected to centrifugation at 3000 rpm for 2–3 min.

Table 1: Age and gender of the subjects						
Group	Gender	Number	Age (in years)			
			Minimum	Maximum	Mean	Standard deviation
Study group	Males	64	11	30	23.25	5.37
	Females	36	11	30	23.11	5.42
	Total	100	11	30	23.25	5.36
Control group	Males	47	12	30	22.9	4.26
	Females	53	12	30	23.31	4.61
	Total	100	12	30	22.40	3.92

Afterward, in a fresh test tube, 10 μl of the saliva was taken to which 1000 μl of glucose reagent was added (provided by the manufacturer). This mixture was incubated (in-built in the semi-auto analyzer) for 10 min at 37°C.

Laboratory investigations

Quantitative measurement of blood and salivary glucose was performed using a semi-auto analyzer [Evolution 3000 By Tulip Diagnostics (P) Ltd, Jaipur, India]. The incubated samples of serum and saliva were analyzed separately. The serum and salivary samples were not preserved, but were directly subject to centrifugation, then incubation, and then glucose analysis without any delay.

Statistical analysis

For the blood and salivary glucose of both the groups, mean and standard deviation were calculated. To check for the statistical significance, z-test was employed which gave a value of $P < 0.05$, that was considered statistically significant for both the study and control groups separately and also when both the groups were considered together. Furthermore, Pearson's correlation was established for the same parameters which came out to be highly significant. Then, linear regression analysis was done between the blood and salivary glucose of the study group and a regression equation was derived. All the statistical analyses were performed using SPSS v16.0 software.

RESULTS

The minimum, maximum, mean, and standard deviation of the blood and salivary glucose values obtained [Table 2]. The statistical values obtained for the comparison of study, control group and inter group comparison is being presented in [Table 3].

Linear regression equation

Linear regression analysis was determined between the blood and salivary glucose levels among the study group and the R-squared (R^2) value obtained was 0.995 [Graph 1]. Further, a regression equation was derived as $y = 9.875x + 4.937$ [blood glucose = 9.875 (salivary glucose) + 4.937] using which a known value of salivary glucose can be converted to blood glucose and vice versa.

Furthermore, a cut-off value of salivary glucose levels was determined for detecting diabetes mellitus. It was observed that with the salivary glucose level greater than 11.60 mg%, the patient is considered as diabetic. Also, the normal range for salivary glucose levels is between 7.60 mg% and 11.60 mg%.

DISCUSSION

The salivary glucose levels likely follow a threshold mechanism. Diabetes mellitus is a heterogeneous metabolic disease characterized by abnormally elevated blood glucose levels.[10] The increase in salivary glucose levels with increase in blood glucose levels has been suggested to be attributed to "leakage" across the basement membrane of the glands.[11]

Whole saliva is frequently studied as an alternative for blood and can be useful for diagnostic purpose. It contains locally produced substances as well as blood components that can be used for diagnosing a variety of systemic diseases and understanding of their oral manifestations.[12,13]

In the present study, for the 200 subjects in the study and control group, correlation was evaluated between the blood and salivary glucose values, which on analysis revealed Pearson's correlation of 0.998 and a P value of <0.01, which was statistically significant.

In a study conducted by Darwazeh *et al.*,[14] statistically significant results were obtained between the blood and the salivary glucose levels when whole saliva was taken into consideration, which are very much similar to the results of our present study.

Jurysta *et al.*[15] conducted a study considering five sets of experiments which consisted of different groups of

Group	Gender	Blood glucose (in mg%)				Salivary glucose (in mg%)			
		Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation
Study group	Males	49	480	205.44	98.63	4.3	48.3	20.23	10.40
	Females	84	377	202.47	94.42	7.8	37.8	19.97	9.66
	Overall	49	480	204.44	96.67	4.3	48.3	20.14	9.86
Control group	Males	64	98	79.83	16.34	5.8	9.28	7.72	0.84
	Females	65	96	80.77	7.29	5.9	9.08	7.52	0.75
	Overall	64	98	82.02	8.00	5.8	9.28	7.65	0.82

Table 2: Minimum, maximum, mean, and standard deviation of the blood and salivary glucose values

Table 3: Correlation between blood glucose and salivary glucose			
Group	Total number of patients	r value	P
Study group	100	0.997	<0.001[a]
Control group	100	0.999	<0.001[b]
Both (study and control groups)	200	0.998	<0.001[c]

[a,b,c]*Statistically significant results obtained when correlating the blood and salivary glucose levels using z–test P<0.05. r value=Pearson's correlation*
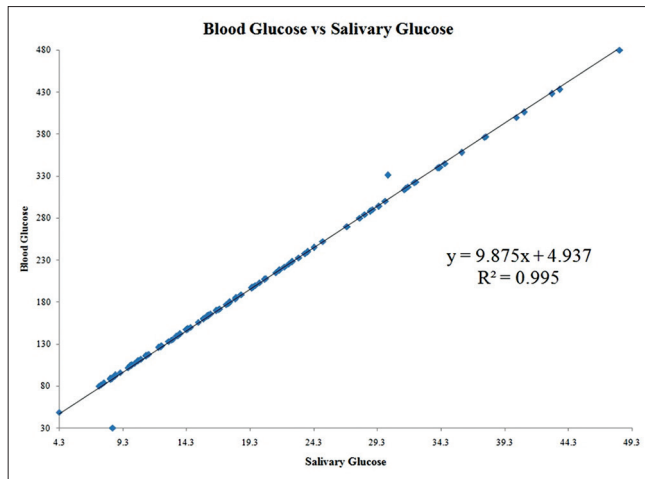
Graph1: Linear regression analysis correlation between blood glucose and salivary glucose

individuals and different methods of analysis of the glucose levels in blood and saliva. They reported that the salivary glucose concentration increased as the blood glucose levels started increasing, which is in accordance with the findings of the present study. Ivanovski *et al.*[16] conducted a study among xerostomic diabetic patients and concluded that there was statistically significant correlation between the blood and salivary glucose levels even if the patient had xerostomia, which is a complication of diabetes.

In the studies carried out by Abikshyeet *et al.*,[17] Panchbhai *et al.*,[18] and Agrawal *et al.*,[19] there was statistically significant correlation between blood and salivary glucose levels when the control group and study group were considered together. In all these studies, the blood and salivary glucose were assessed by glucose oxidase method. The results of all these authors are in accordance with the results of our study.

Statistically non-significant correlations between blood and salivary glucose in the study and control groups were obtained by Tenovuo *et al.*,[20] Aryeh *et al.*,[21] and Vaziri *et al.*,[22] when similar methods for the analysis of blood and salivary glucose were adopted. This variation in result might have been because of the small sample size.

Among the 100 study group subjects, correlation was determined between the blood and salivary glucose values, which on analysis revealed Pearson's correlation value of 0.997. The P value was <0.01, which was statistically significant.

A study conducted by Twetman *et al.*[23] and Belazi *et al.*[24] among children with Type 1 diabetes showed a statistically significant correlation between the blood and the salivary glucose levels, which is in accordance with the present study results.

Results obtained in a study conducted by Gough *et al.*[25] and Guilbault *et al.*[26] were statistically significant, which showed a good correlation between the levels of blood and salivary glucose on using a chromatographic separation method for carbohydrate analysis biosensors respectively, which is in accordance with our present study results.

Andersson *et al.*[27] conducted a study taking into consideration the parotid saliva, which showed a statistically significant result and correlation on comparison of the salivary and the blood glucose levels, and the results are similar to that of the present study. Studies conducted by Shehla *et al.*,[25] Bernardi *et al.*,[28] and Shashikumar *et al.*[11] showed a statistically significant correlation between the salivary and the blood glucose levels, which is accordance with our present study.

The results obtained for blood and salivary glucose levels for the diabetic subjects in the present study are in accordance with the results of a study that was carried out by Lasisi *et al.*[29] who considered diabetes and periodontitis and concluded that there is statistically high correlation between the blood and salivary glucose levels irrespective of the periodontal condition of the patient. When a similar kind of study was carried out by Forbat *et al.*,[30] it revealed a non-significant correlation of blood glucose and salivary glucose levels among the diabetic patients, which is in disagreement with our study. This difference might be because of the different methods used for the analysis of blood and salivary glucose levels and also due to the small sample size.

Among the 100 subjects of the control group, correlation was determined between the blood and salivary glucose levels, which on analysis revealed Pearson's correlation value of 0.999. The P value was <0.001, which was statistically significant.

The above results obtained for the non-diabetic subjects are in accordance with the results of

studies conducted by Abikshyeet *et al.*,[18] Panchbhai *et al.*,[19] and Agrawal *et al.*[20] and the same evaluation mismatched with the study that was conducted by Darwazeh *et al.*[14] which suggested a non-significant correlation between the salivary and blood glucose of control group.

In our study, a cut-off value of salivary glucose levels was estimated for determining diabetes mellitus. It was observed that if the salivary glucose is greater than 11.6 mg%, the patient is considered as diabetic and the normal range of salivary glucose levels is 7.60–11.6 mg%. Also, blood glucose can be predicted for a given salivary glucose level by using the regression equation, i.e. [blood glucose = 9.875 (salivary glucose) +4.937]. The R^2 value obtained using linear regression analysis is 0.995, which is a highly significant.

A similar short study was conducted by Nagalaxmi *et al.*[31] who gave a salivary glucose value of 11.5 mg%, which indicated that above this value, a person may be considered diabetic; this is almost equivalent to the value obtained in our present study.

The statistical correlation in this study has proved that salivary glucose can be used as an indicator for the presence of diabetes, which has an advantage of being a noninvasive method.

Thus, based on the results of the present study, it can be concluded that salivary glucose levels do serve as a reliable indicator of blood glucose levels in the diabetic patients with elevated blood glucose levels.

In the present study, saliva seemed to play a very useful role as a noninvasive method of diabetes monitoring. But further studies need to be undertaken involving other methods to estimate salivary glucose levels, preferably the digital methods, and compare the postprandial and random blood glucose levels and salivary glucose levels, to find the effects of any other systemic disease on the levels of salivary glucose in diabetic patients, and check the effect of use of tobacco products on the salivary glucose levels, to authenticate the role of saliva as a noninvasive method of salivary glucose estimation.

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Conflicts of interest

There are no conflicts of interest.

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