ORIGINAL ARTICLE

Salivary glucose as a diagnostic tool in Type II diabetes mellitus: A case-control study

M Dhanya, S Hegde

Department of Oral Medicine, The Oxford Dental College and Hospital, Bengaluru, Karnataka, India

Abstract

Background and Objectives: The prevalence of diabetes mellitus is increasing steadily in India. Understanding blood glucose level is the key to both diagnosis and management of diabetes mellitus. However, there is an on-going need for improvements in noninvasive, point-of-care tools for the diagnosis and prognosis of diabetes. Assessing a relationship between the blood glucose level and its concentration in other body fluids such as the saliva can help in developing a conservative method for blood sugar assessment replacing venous blood sampling. Diabetes mellitus is known to cause changes in salivary composition. Hence, this study was undertaken to evaluate the relationship of blood glucose level with salivary glucose in diabetic and nondiabetic patients.

Materials and Methods: The study sample included 100 diabetic patients and 100 nondiabetic patients aged above 35 years of age. Fasting blood and salivary glucose levels were measured in the two groups. Pearson's correlation coefficient was used to assess the correlation of blood glucose with salivary glucose in the two groups.

Results: The results of the study revealed an increase in the level of fasting salivary glucose in diabetics compared to that of nondiabetic patients. It also showed a highly significant positive correlation between fasting salivary glucose and serum glucose in both diabetic patients and in controls.

Conclusion: From this study, it can be concluded that fasting salivary glucose level can be used as a noninvasive diagnostic, as well as a monitoring tool to assess the glycemic status of Type II diabetes mellitus patients.

Key words: Fasting salivary glucose, fasting serum glucose, Type II diabetes mellitus

Date of Acceptance: 02-Nov-2015

Introduction

Diabetes mellitus is a clinically and genetically heterogeneous metabolic disease characterized by abnormally elevated hyperglycemia and dysregulation of carbohydrate, protein, and lipid metabolism. The primary feature of diabetes mellitus is chronic hyperglycemia, resulting from either a defect in insulin secretion from pancreas or resistance of body's cells to insulin action or both.

Recent estimates indicate that there were 171 million people in the world with diabetes in the year 2000, and this is projected to increase to 366 million by 2030.[1] According to WHO estimate, 70% diabetics reside in developing countries, and India has world's largest diabetes population with 50.8 million people suffering from diabetes followed

Address for correspondence:
Dr. M Dhanya,
Department of Oral Medicine, The Oxford Dental College and Hospital, Hosur Road, Bommanahalli, Bengaluru, Karnataka, India.
E-mail: dhanya.mukesh1@gmail.com

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/1119-3077.183314

PMID: 27251965

How to cite this article: Dhanya M, Hegde S. Salivary glucose as a diagnostic tool in Type II diabetes mellitus: A case-control study. Niger J Clin Pract 2016;19:486-90.

by China.[2] In 2025, it is approximated that 57.2 million diabetics will be noticed in India.[3]

Type II diabetes mellitus is the fifth most common condition and the sixth leading cause of mortality among the elderly. India is world's second most populous country with a significant number of patients with Type II diabetes.[2] Type II diabetes is associated with serious complications of the eyes, kidneys, heart, blood vessels, and other organ systems. The quality of life is impaired with shortening of the patient's lifespan.[4]

Currently, a diagnosis is achieved by evaluating blood glucose levels. The blood glucose level above 126 mg/dL is diagnosed as diabetes.[5] Monitoring blood glucose at frequent intervals causes unnecessary discomfort and mental trauma to patients and hence a much simpler and noninvasive technique for the diagnosis and monitoring of diabetes is very desirable.

Saliva is the principal defensive factor in the mouth which contains informative components that can be used as diagnostic markers for human disease. A growing number of dental and medical professionals are also finding that saliva provides an easily available, noninvasive diagnostic medium for a rapidly widening range of diseases and clinical situations. The multifarious components within saliva not only protect the integrity of the oral tissues, but also provide clues to local and systemic diseases and conditions.

This study aimed to determine any alterations in the salivary glucose levels and to assess for a correlation between salivary glucose levels and blood glucose levels in both diabetic and nondiabetic patients. Salivary glucose was chosen as a medium to measure blood glucose because saliva is said to be the ultrafiltrate of blood. Glucose is one of the blood components that are transferable across the salivary gland epithelium in proportion to its concentration in blood.

Materials and Methods

The study group comprised of 200 patients aged above 35 years, who visited the outpatient department of the Oxford Dental College and Hospital. Further, the study group was divided as 100 diabetics and 100 nondiabetic aged matched healthy control group. Patients taking treatment for other systemic diseases and pregnant women were excluded from the study group. Written consent was obtained from all the subjects taking part in the study, and a data sheet was completed detailing the person's name, age, sex, and relevant medical history. The subjects were asked to report to the clinic in the morning, on an empty stomach, after 8 h of fasting. Before saliva collection, the patients were asked to rinse their mouth with distilled water and were asked to relax for 5 min. The patients were asked to lean their head forward over the test tube and open their mouth slightly, and saliva was allowed to drain into the tube for about 5 min. Any remaining saliva after 5 min was asked to spit into the test tube.

Following the collection of a saliva sample, 5 ml of venous blood was collected in a sterile test tube. One milliliter of each unstimulated saliva and blood samples was centrifuged separately at 3000 rpm for 20 min, and clear supernatants were processed immediately for estimation of glucose.

Procedure for salivary glucose estimation

Salivary glucose was analyzed by glucose oxidase end point assay using Abcam's Glucose Assay Kit reagents. Three test tubes were taken. Ten microliters of standard solution and 1.0 ml of enzyme reagent was added into one test tube and was marked as "S". In another test tube, 10 μl of saliva sample was pipetted along with 1.0 ml of enzyme reagent. In the third test tube, 10 μl of serum sample was pipetted along with 1.0 ml of enzyme reagent. The contents of all the three test tubes were mixed well and incubated at 37°C for 5 min. The absorbance of the samples was assessed using a colorimeter. The amount of absorbance is proportional to the level of glucose in the saliva and serum.

Statistical analysis

Data were analyzed using statistical software (SPSS (Statistical package for the social sciences) version 12 is a product of IBM, USA). Means and standard deviations (SDs) were calculated for the individual groups. Independent Student's *t*-test ANOVA was used for testing between-groups variation, and Spearman's coefficient was used for measuring the association between variables. A $P < 0.05$ was accepted as a significant and a value < 0.01 was considered highly significant.

Results

The mean age of patients in the study group was 53.01 and that in the control group was 52.42. The mean serum glucose in diabetic group was 136.30 mg/dL with an SD of 28.58. In the control group, the mean serum glucose level was 97.78 mg/dL and SD was 6.39. Comparisons of blood glucose levels between the control and diabetic groups revealed that the difference was highly significant ($P = 0.00001$) [Table 1]. The mean salivary glucose in diabetic group was 8.47 mg/dL with an SD of 4.20. In the control group, the mean salivary glucose level was 1.20 mg/dL and SD was 0.86 [Table 2]. Comparisons of salivary glucose levels between the control and diabetic groups showed that the difference was highly significant ($P = 0.00001$).

The patients were subdivided into three subgroups depending on their serum glucose levels. The salivary glucose levels of the three subgroups were compared using one-way ANOVA, and the difference was found to be highly

Table 1: Comparison of controls and diabetics with blood glucose levels

Group	Mean	SD	*t*-value	*P* value
Control	97.78	6.39	−13.1522	0.00001*
Diabetics	136.30	28.58		

*=*P*<0.01. SD=Standard deviation

Table 2: Comparison of controls and diabetics with salivary glucose levels

Group	Mean	SD	*t*-value	*P* value
Control	1.20	0.86	−16.9524	0.00001*
Diabetics	8.47	4.20		

*=*P*<0.01. SD=Standard deviation

Table 3: Comparison of blood glucose levels with salivary glucose in diabetics

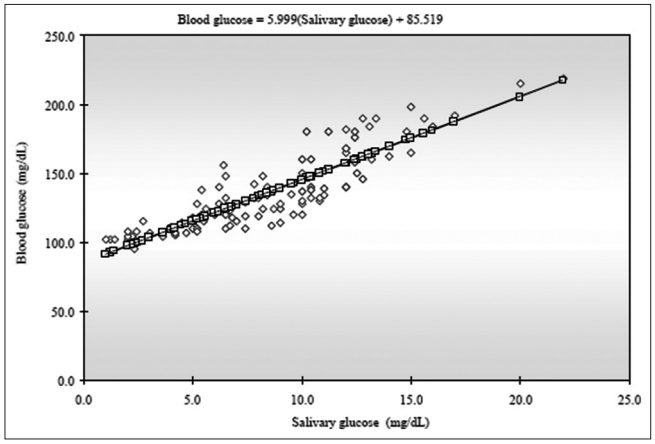
	Salivary glucose levels	
	Means	SD
Blood glucose levels		
Below 130 mg/dL	5.43	2.53
130-200 mg/dL	10.99	2.72
Above 200 mg/dL	21.00	1.41
Total	8.47	4.20
F	78.5798	
P	0.00001*	
Pair wise comparison by Tukeys multiple *posthoc* procedures		
Below 130 mg/dL versus 130-200 mg/dL	*P*=0.0001*	
Below 130 mg/dL versus Above 200 mg/dL	*P*=0.0001*	
130-200 mg/dL versus above 200 mg/dL	*P*=0.0008*	

*=*P*<0.01. SD=Standard deviation

Table 4: Correlations among blood glucose levels and salivary glucose levels

Variables	Salivary glucose level in controls	Salivary glucose level in diabetics
Blood glucose level in controls	*R*=0.6342*	
Blood glucose level in diabetics		*R*=0.8809*

*=*P*<0.01



Graph 1: Correlation between salivary and blood glucose levels in the diabetic group

significant ($P < 0.01$). Further, to evaluate the difference between the groups a Tukey's multiple *post-hoc* procedure was performed. The results showed that each group had a statistically significant difference ($P < 0.01$) in the salivary glucose levels [Table 3].

The correlation coefficient between serum glucose and salivary glucose was calculated in both diabetic patients, and healthy controls and the *r* value was found to be 0.8809 and 0.6342, respectively, which were statistically significant ($P < 0.05$) [Table 4].

Discussion

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.[6] National Urban Diabetes Survey Study done in 2001 showed that Bengaluru had 12.4% prevalence of Type II diabetes.[7]

Blood glucose monitoring by the patient and the physician is an important aspect in the management of diabetes in order to control the debilitating complications of the disease. Conventional monitoring techniques require blood sample and the invasive procedures often cause pain and discomfort which may limit frequent testing.

With ever improving advances in diagnostic pathology, the race for the next generation of bloodless, painless, and accurate glucose instruments has begun. Hence, the other biological fluids like urine, sweat, and saliva are also being utilized for the diagnosis of diabetes mellitus. Of these, saliva offers distinctive advantages because it can be collected noninvasively by individuals with modest training. Furthermore, saliva may provide a cost-effective approach for the screening of large populations.

The mean age of the study group was 53.01, which was higher than that reported by Prathibha *et al*.[8] and Panchbhai *et al*.[9] where the mean age was 46.5 years and 48.5 years, respectively. These data highlights the occurrence of Type II diabetes mellitus in a relatively younger age group, especially in developing countries as reported by Chennai Urban Rural Epidemiology Study.[10]

In this study, the mean serum fasting glucose levels in diabetic group were 136.30 mg/dL with SD being 28.58. The mean serum glucose in controls was 97.78 with SD of 6.39.

In this study, salivary glucose concentration of diabetic and nondiabetic individuals was analyzed. Among the diabetic patients mean salivary glucose levels in fasting state was 8.47 mg/dL with SD of 4.20. In comparison, the control group had a lower mean salivary glucose of 1.20 mg/dL with an SD of 0.86. Maximum salivary glucose in control

group level did not exceed 5 mg/dL which was much lesser than least values of diabetic patients. Similar to our study, Mahdavi *et al.*[11] have reported the presence of sugar in the saliva of diabetic patients and many other authors including Panchbhai *et al.*,[9] Priya *et al.*,[12] Amer *et al.*,[13] Agrawal *et al.*,[14] Abikshyeet *et al.*,[15] and Ivanovski *et al.*[16] have also reported increases in salivary glucose levels in diabetes mellitus patients in comparison to nondiabetics. However, contradictory to our study Bakianian Vaziri *et al.*[17] concluded that there was no statistically significant difference in salivary glucose concentrations between diabetic patients and control patients. Similarly, Carda *et al.*[18] concluded that the salivary glucose levels of 76.4% of diabetic patients were in the normal range.

In this study, there was a positive correlation between salivary and serum glucose in diabetic patients, as well as the controls. These correlations were found to be statistically significant. Hence, it can be stated that salivary glucose can be used as an indicator of serum glucose concentration in diabetic patients. The results of our study were in accordance with the study conducted by Abikshyeet *et al.*,[15] Amer *et al.*,[13] Agrawal *et al.*,[14] and Panchbhai *et al.*[9] Our study had a slightly higher positive correlation of about 0.8809 in diabetes patients and 0.6342 in controls compared to the other similar studies. However, in contrast to our study, the studies by Jurysta *et al.*,[19] Carda *et al.*,[18] and Vasconcelos *et al.*[20] failed to establish a correlation between salivary glucose and serum glucose and they concluded that salivary glucose is not directly influenced by glycemia and hence cannot be used to monitor glycemic control in diabetics. Again these contradictory results may be due to the difference in the selection criteria and the study method. Moreover, researchers from Brown University have developed a new biochip sensor that can selectively measure concentrations of glucose in human saliva and they concluded that it was an important step that would enable people with diabetes to test their glucose levels without drawing blood.[21]

In this study, we divided the diabetic group into three subgroups based on their serum glucose levels. We compared the salivary glucose levels of these three subgroups. On comparing the subgroups, a statistically significant difference in salivary glucose was observed in the three groups. The study showed that patients with serum glucose levels below 130 mg/dL had a mean salivary glucose of 5.43; patients with serum glucose levels between 130 and 200 mg/dL had a mean salivary glucose of 10.99 mg/dL and patients with serum glucose level above 200 mg/dL reflected a mean salivary glucose of 21.00 mg/dL. From these data, it can be noted that salivary glucose above 5.5 mg/dL has a high chance of having serum glucose levels above 130 mg/dL. Abikshyeet *et al.*[15] in their study concluded that salivary glucose levels above 4 mg/dL have a very high chance of having serum glucose levels above 130 mg/dL. Due to method variation and the population selected a slight variation is noticed from this study.

In this study, a highly significant positive correlation wasfound between salivary glucose and serum glucose in both diabetic patients and in controls, and a regression coefficient was calculated [Graph 1]. The regression coefficient gives the amount of increase or decrease in the serum glucose for a unit change in the salivary glucose. Hence, from a given value of salivary glucose, we can predict serum glucose level by using the regression equation. The respective formulae are:

Serum glucose = 5.999 (salivary glucose) + 85.519 in diabetic group
= 4.7254 (salivary glucose) + 92.095 in control group.

Since regression formulas derived for serum glucose shows to be population specific as the mean age and SD varied according to the populations involved, we recommend further studies involving large samples in different populations.

From this study, it can be inferred that glucose can be detected in saliva of both diabetic and nondiabetic patients and the fact that the salivary glucose levels are higher in diabetics compared to that of healthy controls. The assessment of glucose levels to detect diabetes noninvasively will facilitate interventions designed to prevent or delay progression to uncontrolled diabetes and its complications.

Limitations of the study

A lot of factors influence the secretion of saliva and the constituents of saliva. Since raised glucose level is also recorded in gingival crevicular fluid, the glucose in the saliva may not be exclusively of salivary gland origin. Hence, the technique we adopted requires validation and future studies has to be concentrated on glucose estimation from specific glandular saliva.

Conclusion

This study highlights the possibility of using saliva as a diagnostic tool to assess the glucose concentration in blood, thereby making self-measurement of glucose less invasive. From our study, it could be concluded that glucose was detectable in the saliva of diabetic and nondiabetic individuals. A significant positive correlation was established between blood glucose and salivary glucose levels. From this study, an equation was formulated to assess the serum glucose level, utilizing the measured salivary glucose level. Hence, it can be concluded that fasting salivary glucose level can be used as a noninvasive diagnostic, as well as a monitoring tool to assess the glycemic status of diabetes

mellitus patients. This study highlights the role of dentists in diagnosing diabetes mellitus via salivary samples and hence promoting health for all in the society.

Further research should be focused on the correlation between salivary and serum glucose with a standardized technique. It is possible that tests based on saliva can have a substantial role in diagnosis and also early detection of diabetes mellitus. Moreover, further research has to be done to know the standard range of salivary glucose levels for diagnosing diabetes mellitus.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Diabetes facts. In: IDF Diabetes Atlas. 6th ed. 2013. Available from: http://www.idf.org/diabetesatlas. [Last accessed on 2014 Dec 12].
3. Pendsey S. Gravity of problem. In: Practical Management of Diabetes. 2nd ed. New Delhi: Jaypee Brothers; 2004. p. 7-12.
4. Little RR, Goldstein DE. Endocrine (standardization of glycohemoglobin measurement). Anal Chem 1995;67:393R-7R.
5. American Diabetes Association. Exclusive summary standards of medical care in diabetes. Diabetes Care 2012;35:4-10.
6. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J 2014;7:45-8.
7. Mohan V, Pradeepa R. Epidemiology of diabetes in different regions of India. Health Adm 2009;22:1-18.
8. Prathibha KM, Johnson P, Ganesh M, Subhashini AS. Evaluation of salivary profile among adult type 2 diabetes mellitus patients in South India. J Clin Diagn Res 2013;7:1592-5.
9. Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Sci 2010;52:359-68.
10. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 2007;125:217-30.
11. Mahdavi SO, Hashemi S, Boostani NS, Zokaee H. A new method to evaluate fasting plasma glucose by salivary glucose measurement. Iran J Diabetes Obes 2012;4:127-32.
12. Priya SS, Bharani GO, Nagalingam M, Jayanthi M, Kanagavalli U. Potential of salivary protein as a biomarker in prognosis of diabetes mellitus. J Pharm Res 2011;4:2228-9.
13. Amer S, Yousuf M, Siddqiui PQ, Alam J. Salivary glucose concentrations in patients with diabetes mellitus – A minimally invasive technique for monitoring blood glucose levels. Pak J Pharm Sci 2001;14:33-7.
14. Agrawal RP, Sharma N, Rathore MS, Gupta VB, Jain S, Agarwal V, *et al*. Noninvasive method for glucose level estimation by saliva. J Diabetes Metab 2013;4:266.
15. Abikshyeet P, Ramesh V, Oza N. Glucose estimation in the salivary secretion of diabetes mellitus patients. Diabetes Metab Syndr Obes 2012;5:149-54.
16. Ivanovski K, Naumovski V, Kostadinova M, Pesevska S, Drijanska K, Filipce V. Xerostomia and salivary levels of glucose and urea in patients with diabetes. Prilozi 2012;33:219-29.
17. Bakianian Vaziri P, Vahedi M, Mortazavi H, Abdollahzadeh SH, Hajilooi M. Evaluation of salivary glucose, IgA and flow rate in diabetic patients: Acase-control study. J Dent (Tehran) 2010;7:13-8.
18. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal 2006;11:E309-14.
19. Jurysta C, Bulur N, Oguzhan B, Satman I, Yilmaz TM, Malaisse WJ, *et al.* Salivary glucose concentration and excretion in normal and diabetic subjects. J Biomed Biotechnol 2009;2009:430426.
20. Vasconcelos AC, Soares MS, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. J Oral Sci 2010;52:293-8.
21. Progress on Detecting Glucose Levels in Saliva. Phys.org. June 3, 2014. Available from: http://www.phys.org/news/2014-06-glucose-saliva.html. [Last accessed on 2014 Dec 12].