ORIGINAL RESEARCH

Correlation of Blood Glucose Levels, Salivary Glucose Levels and Oral Colony Forming Units of *Candida albicans* in Type 2 Diabetes Mellitus Patients

Nitu Mishra[1], Aparna Trivedi[2], Sajda K Gajdhar[3], Harshad Bhagwat[4], Gaurav K Khutwad[5], Prashant E Mall[6], Dinraj Kulkarni[7]

Abstract

Aim: The study aims to correlate salivary glucose, blood glucose levels and oral colony forming units of *Candida albicans* and to evaluate whether saliva can be used as noninvasive means to measure glycemic status in type II diabetics without the need for the invasive procedure.

Materials and methods: The study included 100 type II diabetic patients (group I) of both genders with age 40 years and above and100 healthy patients (group II), age and sex matched with the study group. Group I includes uncontrolled and controlled diabetics as groups IA and IB, respectively. Salivary glucose measurement was done using the enzymatic colorimetric method and blood glucose levels measured by doing venepuncture and centrifuged. The oral candidal carriage was calculated by incubation in Sabouraud's dextrose agar supplemented with chloramphenicol and incubated aerobically for 48 hours. To compare the mean values Z test was applied. To determine the relationship between two variables Pearson's correlation coefficient was used.

Results: The salivary glucose levels showed a significant correlation with blood glucose levels. The salivary candida carriage was higher in uncontrolled as compared to controlled diabetics and healthy individuals.

Conclusion: Positive correlation was obtained between salivary glucose and blood glucose in diabetics and candidal carriage has a positive correlation with blood glucose levels. This salivary glucose and blood glucose levels correlation confirms its use to find glycemic status in diabetic patients.

Clinical significance: The positive correlation of salivary glucose with blood glucose shows that it can be utilized as a noninvasive tool for monitoring glycemic status in diabetic patients.

Keywords: Diabetics, Glucose oxidase kit, Oral candidal carriage, Salivary glucose, Sabouraud's dextrose agar.

The Journal of Contemporary Dental Practice (2019): 10.5005/jp-journals-10024-2545

Introduction

Diabetes mellitus (DM) is a disorder characterized by a relative or complete insufficiency of insulin secretion or resistance to metabolic action of insulin on target tissues.[1]

The classification scheme for diabetes mellitus includes two major forms: type I (insulin dependent diabetes mellitus/IDDM) and typeII(noninsulin dependent diabetes mellitus/NIDDM). Type II includes the most common form of diabetes, which combines insulin resistance with an insulin secretory defect. Patients with type II DM have some endogenous insulin secretory capability but have overt abnormalities of glucose homeostasis, including fasting hyperglycemia.[2]

There is variable and at times, intense effects of diabetes on the oral tissues. Diabetic patients are more prone to severe and recurrent oral infections. Studies prove a remarkable correlation between salivary and blood glucose levels in DM.[1]

Glucose is a small molecule that diffuses easily through the membrane of the blood vessels, passing through the bloodstream to the gingival fluid, by way of the gingival sulcus, and making its way to the saliva.[3]

Candida albicans is the most prevalent candida species isolated either from the oral cavity of diabetic patients and healthy individuals. The frequency and density of candida colonization and the development of oral candidosis in diabetes mellitus patients seem more the result of a combination of host and fungal risk factors rather than any other single factor.[2]

The level of some components of saliva may be related to certain systemic illness depicting the patient's nutritional, hormonal and metabolic status. Saliva is an organic fluid. It is easy to collect saliva by noninvasive means, and it can be preserved easily.[1]

[1,2]Department of Oral Pathology and Microbiology, Modern Dental College and Research Center, Indore, Madhya Pradesh, India

[3]Department of Basic Dental Clinical Sciences, IBN Sina National College for Medical Studies, Jeddah, Kingdom of Saudi Arabia

[4-7]Department of Oral and Maxillofacial Surgery, MA Rangoonwala College of Dental Sciences and Research Center, Pune, Maharashtra, India

Corresponding Author: Nitu Mishra, Department of Oral Pathology and Microbiology,, Modern Dental College and Research Center, Indore, Madhya Pradesh, India, Phone: +919406606099, e-mail: drnitumishra@gmail.com

How to cite this article: Mishra N, Trivedi A, Gajdhar SK, Bhagwat H, Khutwad GK, Mall PE, Kulkarni D. Correlation of Blood Glucose Levels, Salivary Glucose Levels and Oral Colony Forming Units of *Candida albicans* in Type 2 Diabetes Mellitus Patients. J Contemp Dent Pract 2019;20(4):494-498.

Source of support: Nil

Conflict of interest: None

The aim of the present study was to assess the potential of saliva in monitoring DM. The objectives of the study were to correlate salivary glucose levels, blood glucose levels, and oral candida carriage and to assess whether saliva can be used as a noninvasive and useful tool for monitoring diabetes.

Materials and Methods

This study was conducted in the Department of Oral Pathology and Microbiology, Modern Dental College and Research Centre,

Indore. Ethical clearance for the study was obtained from scientific research committee of Devi Ahilyabai Vishvavidyalaya, Indore, Madhya pradesh, India. Participants were informed about the study protocol, and blood investigations were done to identify both the groups and only those who provided their written consent were included in the study.

Inclusion Criteria

Hundred patients previously diagnosed with type II DM preferably above 40 years of age, with no other systemic illness, and 100 healthy individuals with age 40 years and more with no apparent medical history.

Exclusion Criteria

- Pregnant women
- Patients on radiotherapy
- Patients on any medications other than for type 2 DM
- Subjects with any oral mucosal lesions

The study population comprised two groups:

- Group I consisted of 100 type II diabetic patients of both genders with age 40 years and above. It was again subdivided into group I (A) in which there were 45 uncontrolled diabetic patients and group I (B) in which there were 55 controlled diabetic patients.
- Group II includes a control group comprised of 100 healthy/nondiabetic patients.

A detailed case history was taken regarding the duration of diabetes, regular medications and medications on the day of sample collection followed by a general and oral examination.

Blood Glucose Measurement

Two mL blood samples were obtained from the cephalic vein using a 24-gauge needle, and the sample was immediately transferred into 2 mL fluoride oxalate. The sample was immediately centrifuged at 2000 rpm, and 10 µL of plasma was added to 1 mL of test reagent and incubated at 37°C for 10 minutes, and the absorbance values were read in a semi-automated analyzer (Fig. 1).

Salivary Glucose Measurement (Enzymatic Colorimetric Test Method)

Patients had their morning breakfast along with medication 2 hours before the sample collection."Spit Technique" was used to collect unstimulated saliva. Sitting upright on the dental chair with the head kept forward, the patient was instructed not to swallow, speak, or do any movements of the head. The saliva was collected in a graduated sterile container every 5 minutes. Glucose oxidase kit was used in semiautomated analyzer to get the glucose levels of unstimulated saliva (Fig. 2). One thousand µL of the reagent was mixed with 10 µL of the sample and incubated at 37°C for 10 minutes. Absorbance values of sample and standard against reagent blank were measured at 510 nm. The color was made stable for 30 minutes at room temperature.

Mycological Investigations

After salivary glucose estimation did the subjects were requested to rinse the mouth with 10 mL phosphate buffer saline for 60 seconds. The sample was then centrifuged at 3000 rpm for 15 minutes. The deposit was inoculated onto Sabouraud's dextrose agar supplemented with Chloramphenicol and then incubated aerobically at 37°C for 48 hours. CFU count was multiplied by 1000 to get CFU/mL (Figs 3 and 4).

Statistical Analysis

Mean and standard deviations (SDs) were calculated for individual groups. To compare the mean values z-test was applied for differences of two sample means as sample size n >30 in all the groups. To determine the relationship between two variables Pearson's correlation coefficient was used.

Results

Though mean salivary and blood glucose levels showed statistically significant differences between normal subjects and controlled diabetics (p <0.001) (group II *vs.* group IB), mean salivary colony forming units of C. albicans were found to be nonsignificant (p >0.05) (Table 1).

When normal subjects were compared with uncontrolled diabetics (group II *vs.* group IA), all three parameters showed statistically significant differences (p <0.01); values being higher in group IA (Table 2).

Similar trends were reported when controlled and uncontrolled diabetics were compared (group IB and IA); values being higher in group IA (Table 3).

In normal subjects, all parameters showed statistically highly significant correlations (p <0.01).

In controlled diabetics, a highly significant correlation (p <0.01) was found between mean blood and salivary glucose levels and mean blood glucose levels and colony forming units of Candida.

Fig. 1: Centrifuge machine

Fig. 2: Semiautomated analyzer

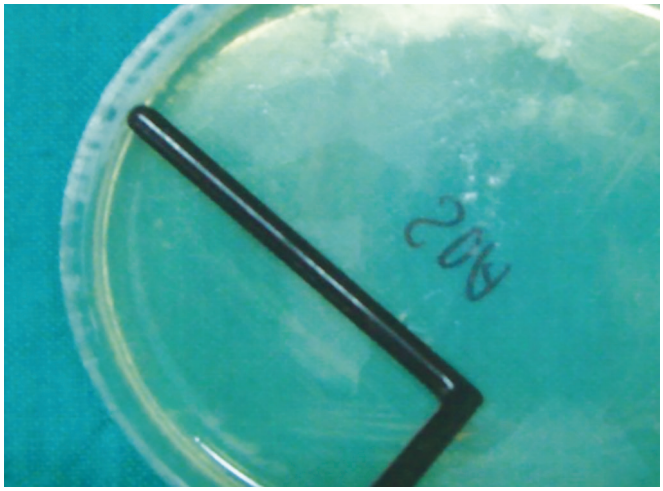

Fig. 3: Inoculating loop and petridish

Fig. 4: Candidal colonies in SDA medium

Table 1: Comparison of various parameters in groups II and IB

Parameters	*Normal subjects (Group II)*	*Controlled diabetics (Group IB)*	*Significance (p value)*
Mean salivary glucose levels (mg/dL)	3.33 ± 1.14	4.96 ± 2.04	*p* <0.001 (HS)
Mean blood glucose levels (mg/dL)	94.32 ± 12.53	142.02±31.17	*p* <0.001 (HS)
Mean colony forming units of *Candida albicans* (CFU/mL)	611 ± 1382.02	1065.45 ± 2182.34	*p* >0.05 (NS)

Table 2: Comparison of various parameters in groups II and IA

Parameters	*Normal (Group II)*	*Uncontrolled diabetics (Group IA)*	*Significance (p value)*
Mean salivary glucose levels	3.33 ± 1.14	9.34 ± 2.99	*p* <0.001 (HS)
Mean blood glucose levels	94.32 ± 12.53	273.09 ± 54.15	*p* <0.001 (HS)
Mean colony forming units of *Candida albicans* (CFU/mL)	611±1382.02	5033.33±2821.11	*p* <0.001 (HS)

Table 3: Comparison of various parameters in group IB and group IA

Parameters	*Controlled diabetics (Group IB)*	*Uncontrolled diabetics (Group IA)*	*Significance (p value)*
Mean salivary glucose levels	4.96 ± 2.04	9.34 ± 2.99	*p* <0.001 (HS)
Mean blood glucose levels	142.02 ± 31.17	273.09 ± 54.15	*p* <0.001 (HS)
Mean colony forming units of *Candida albicans* (CFU/mL)	1065.45 ± 2182.34	5033.33 ± 2821.11	*p* <0.001 (HS)

The nonsignificant correlation was observed in salivary glucose and candida CFUs (*p* >0.05).

In uncontrolled diabetics, a significant correlation (*p* <0.05) was found between mean blood and salivary glucose levels and mean blood glucose levels and colony forming units of Candida. The nonsignificant correlation was observed in salivary glucose and candida CFUs (*p* >0.05) (Table 4).

Discussion

Dentists can counsel their patients with diabetes about improving glucose regulation, maintaining oral and nutritional health, performing daily glucose monitoring tests and seeing a medical professional for routine care.[4] However the whole saliva is frequently studied as an alternative for blood that can be useful even for diagnostic purposes.[5] Noninvasive collection and cost-effectiveness for screening large populations are the advantages of salivary assessment.[5] Saliva play a key role in maintaining homeostasis of the oral cavity by stabilizing the ecosystem of the oral cavity, therefore, serves as one of the markers for successful treatment and risk estimation of the disease.[6,7] Saliva is the ultrafiltrate of blood. Glucose being one of the blood components is easily transferable across the salivary gland epithelium in proportion to its concentration in blood. Saliva is simple to collect.[7] Normal glucose levels in saliva are 0.5–1.00 mg/100 mL and it do not support the growth of microorganisms. Biochemical test show that the normal value of salivary glucose in a healthy nondiabetic individual is <2 mg/dL.[8]

In DM patients there is often increased the presence of Candida species and the density of candidal growth.[9] Several studies have reported that the prevalence of yeast carriage among patients with diabetes could reach up to 54% and that *C. albicans* could account for 25–69% of the isolates.[10] The aim of the study was to find a correlation between blood glucose, salivary glucose levels and oral colony forming units of *C. albicans* in type II diabetic patients. The results of our study show an increase in the salivary glucose levels in uncontrolled (9.34 ± 2.99 mg/dL) and controlled diabetics (4.96 ± 2.04 mg/dL) as compared with normal patients (3.33 ± 1.14 mg/dL). These findings are similar to the findings of Andersson et al. who also

Table 4: Correlation coefficients between various parameters

Groups	*Parameters*	*Correlation coefficients (r)*	*Significance (p value)*
Normal subjects (Group II)	Mean blood glucose levels and mean salivary glucose levels	0.566	p <0.01 (HS)
	Mean blood glucose levels and mean colony forming units of *C. albicans*	0.3	p <0.01 (HS)
	Mean salivary glucose levels and mean colony forming units of *C. albicans*	0.618	p <0.01 (HS)
Controlled diabetics (Group IB)	Mean blood glucose levels and mean salivary glucose levels	0.43	p <0.01 (HS)
	Mean blood glucose levels and mean colony forming units of *C. albicans*	0.36	p <0.01 (HS)
	Mean salivary glucose levels and mean colony forming units of *C. albicans*	0.235	p >0.05 (NS)
Uncontrolled diabetics (Group IA)	Mean blood glucose levels and mean salivary glucose levels	0.3	p <0.05 (S)
	Mean blood glucose levels and mean colony forming units of *C. albicans*	0.347	p <0.05 (S)
	Mean salivary glucose levels and mean colony forming units of *C. albicans*	0.11	p >0.05 (NS)

observed that salivary glucose levels were higher in diabetic subjects than in nondiabetic subjects.[11] Similar results were also found in the study conducted by Panchbhai et al. confirming higher mean salivary glucose uncontrolled and controlled diabetics group than in the healthy nondiabetic group and the differences were highly significant (p <0.001).[12] The mean blood glucose levels in uncontrolled diabetics, controlled diabetics, and normal patients were (273.09 ± 54.15 mg/dL), (142.02 ± 31.17 mg/dL) and (94.32 ±12.53 mg/dL), respectively. The salivary glucose concentrations seem to correlate with the serum glucose concentration in the patients of diabetes mellitus as also indicated earlier by Amer et al.[13] Significant positive correlation was found by Shashikumar et al. between random fasting plasma glucose and salivary glucose in normal and uncontrolled diabetic subjects. This correlation is due to collection of whole mouth fluid in which the raised glucose levels are not only due to leakage across the basement membrane of major and minor salivary glands but potentially also from the gingival crevicular fluid.[1] The mean value of CFUs were 5033.33 ± 2821.11, 1065.45 ± 2182.34 and 611 ± 1382.02 in uncontrolled diabetics, controlled diabetics and normal patients respectively.A study conducted by Safia et al. also showed a higher frequency of candidal growth in diabetic patients.[9] A similar study conducted by `Khaled Abu Elteen also showed that positive yeast in 58.3% of diabetics compared with 30% in healthy controls. This is also in agreement with numerous previous studies which have all indicated that diabetes mellitus enhances candida colonization and proliferation.[10] Similar results were found by Jones et al.[14] and Kumar et al.[15] Safia et al. conducted a study in which they have concluded that the carriage rate of candida was significantly higher in diabetics subjects than in the controls.[9] Study done by Shashikumar et al. concluded that there was a positive correlation between salivary glucose and Candida CFU in overall study population confirming the results of Darwazeh and Kadir et al.[1,16]

The permeability of parotid gland basement membrane is higher in diabetes mellitus, causing raised percolation of glucose, amylase, and protein from the blood thus raising their levels in saliva. This membrane permeability is explained by diabetic membranopathy.[12] There is a controversy existing between the concentration of glucose in the sera and the salivary fluid. The poor correlation prevailing in diabetic patients could be due to many factors like glucose utilization by bacteria, oral retention of alimentary carbohydrates and release of carbohydrates from salivary glycoproteins.[17] Abikshyeet et al. formulated equations to predict fasting sera glucose levels and HbA1c percentage when fasting salivary glucose levels were known. However, accurate sera glucose levels could not be calculated by such equations in all the patients.[18] The other limitations of saliva in diagnosis as well as in the regular monitoring of diabetic patients includes numerous autoimmune inflammatory conditions like Sjogren syndrome and primary biliary cirrhosis, granulomatous conditions including sarcoidosis, degenerative diseases like amyloidosis, graft-versus-host disease, malignant conditions like lymphomas, infections including HIV/AIDS, hepatitis C, and salivary gland agenesis or aplasia apart from drug-induced xerostomia and the total solids seen in the saliva change to the extent of not being reliable for diagnostics as well as in the regular monitoring of the patients. Patients with salivary gland changes after radiation exposure in the head and neck area also pose such challenges.[17]

Conclusion

The present study founds a positive correlation between salivary glucose and blood glucose levels in diabetics and the saliva can be used as a noninvasive means to monitor glycemic status in diabetics. The CFUs of *Candida* has a positive correlation with blood glucose however the correlation between salivary glucose and CFUs of *Candida* is nonsignificant in the diabetic group and significant in the nondiabetic group. Therefore, this study concludes that salivary glucose can be used to assess the diabetic status of the patients and also can be used to detect new cases of diabetes. The candidal carriage is higher in patients with higher blood glucose. The direct correlation between salivary glucose and candidal carriage could not be drawn as the later depends upon several local and systemic factors.

Clinical Significance

Saliva offers some distinctive advantages, greater sensitivity, noninvasive and easy collection procedure. Also, can be collected anywhere, no trained personnel required, good patients cooperation and cost-effective. Hence can be used as noninvasive means to find glycemic status in diabetics.

References

1. Sashikumar R, Kannan R. Salivary glucose levels and oral candidal carriage in type II diabetics. Oral Surgery, Oral Medicine Oral Pathology, Oral Radiology and Endodontology 2010;109(5):706-711.
2. Manfredi M, Cullough MJ, Vescovi P, et al. Updates on diabetes mellitus and related oral diseases. Oral Diseases 2004;10: 187-200.
3. Soares MSM, Batista- Filho MMV, Pimentel MJ, et al. Determination of salivary glucose in healthy adults. Med Oral Patol Oral Cir Buccal 2009;14(10):e510-e513.
4. Ship JA. Diabetes and oral health, an overview. Journal of American Dental Association 2003;134:4s-10s.
5. Vaziri PB, Vahedi M, Mortazavi H, et al. Evaluation of salivary glucose, IgA and flow rate in diabetic patients: a case control study. Journal of Dentistry 2010;7(1):13-18.
6. Elkafri IH, Mashlah A, Shaqifa A. Relationship between blood glucose levels and salivary pH and buffering capacity in type II diabetes patients. East Mediterr Health J 2014;20:139-45.
7. Panchbhai AS. Correlation of salivary glucose level with blood glucose level in diabetes mellitus. J Oral Maxillofac Res. 2012;3:e3.
8. Carda C, Mosquera-Lloreda N, Salom L, et al. Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal. 2006;11:E309-E314. [PubMed]
9. AL-Attas A, Amro SO. Candidal colonization, strain diversity and antifungal susceptibility among adult diabetic patients. Ann Saudi Med 2010 Mar-Apr;30(2): 101-108.
10. Abu-Elteen KH, Hamad MA, Salah SA. Prevalence of oral candida infections in diabetic patients. Bahrain Medical Bulletin 2006; 28 (1): 1-8.
11. Borg Andersson A, Birkhed D, Berntorp K, et al. Glucose concentration in parotid saliva after glucose/food intake in individuals with glucose intolerance and diabetes mellitus. Eur J Oral Sci1998;106:931-937
12. Panchbhai AS, Degewekar SS, Bhowte R. Estimation of salivary glucose, salivary amylase, salivary total protein, salivary flow rate in diabetics in India. Journal of Oral Science 2010;52:359-368.
13. Amer S, Yousef M, Siddique PQ, et al. Salivary glucose concentration in patients with diabetes mellitus - a minimally invasive technique for monitoring blood glucose levels. Pak J of Pharm Sci Jan 2001;14(1):33-37.
14. Tapper-Jones LM, Aldred MJ, Walker DM, Hayes TM. Candidal infections and populations of candida albicans in mouths of diabetics. J Clin Pathol 1981; 34:706-711.
15. Kumar BV, Padshetty NS et al. Prevalence of candida in the oral cavity of diabetic subjects. Journal of Biomedicine & Biotechnology 2009; 10:11-55
16. Darwazeh AM, MacFarlane TW, McCuish A, et al. Mixed salivary glucose levels and candidal carriage in patients with diabetes mellitus. J Oral Pathol Med 1991;20:280-283.
17. Kartheeki B, Abhishek S N, Ravikiran A, et al. Serum and salivary glucose levels in diabetes mellitus: A Review. Nigerian J of general practice 2017;15(2):17-21.
18. Abikshyeet P, Ramesh V, Oza N. Glucose estimation in the salivary secretion of diabetes mellitus patients. Diabetes Metab Syndr Obes 2012;5:149-154.