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A Comparative study on Glucose Levels in Serum and Saliva of Patients with Diabetes Mellitus and Healthy Individuals in Mullana Area

Sonam Bhalla,[1*] Asha Karadwal,[1], Swati Roy,[1] Vivek Dahiya[2]

[1]*Oral Maxillofacial Pathology & Microbiology, YIDSR, Gadholi, Yamunanagar, Haryana (India),*

[2]*Oral Maxillofacial Pathology & Microbiology, MMCDSR, MMU, Ambala, Haryana (India).*

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ABSTRACT

The present study intends to compare and correlate serum & salivary glucose level in healthy & diabetic individuals. A total of 500 subjects were examined, of which 429 were healthy & 71 were confirmed diabetics. Post–prandial blood and salivary samples were analyzed. Mann-Whitney test using Pearson correlation was applied, which was statistically significant ($P < 0.001$). To conclude, glucose concentration in saliva is higher in diabetics than in healthy individuals. Moreover, the changes in serum glucose concentration are followed by the changes in salivary glucose concentration. Thus, saliva can be used as an adjunct diagnostic tool in diabetes mellitus

Keywords: Saliva, Salivary glucose, Diabetes Mellitus, Random Plasma Glucose, Non invasive diagnostic tool

INTRODUCTION

Diabetes Mellitus is the most common endocrine metabolic disorder with incidence of 20.2 per 1000 persons per year in India and 1.9 million new cases diagnosed per year worldwide. Self measurement of glucose is an integral part of Diabetes Mellitus therapy. The diagnostic test for Diabetes has been for years, the invasive technique of blood withdrawal. Therefore the need is to develop a simple method of minimally invasive glucose monitoring. Glucose is a small molecule that diffuses easily through the vessel wall, passing through the blood to the gingival fluid by way of gingival sulcus and to saliva. Moreover saliva is easy to collect by non-invasive methods. Therefore in order to explore the use of saliva as non-invasive diagnostic tool for monitoring, the present study is aimed at evaluating the relation between serum glucose level and salivary glucose level in diabetics.[1,2,3]

MATERIALS AND METHODS

The base sample comprised of 500 healthy individuals in the age group ranging from 8-95 years, who came for regular haematological investigations in the department of Oral and Maxillofacial Pathology in M.M Institute of Dental Sciences & Research, Maharishi Markandeshwar University, Mullana, Ambala (Haryana). The study was conducted in the year 2012-2013 and proceeded with the approval of ethical committee. An informed consent was taken from the subjects of the study and then blood and whole saliva samples were collected from them during resting conditions.

Under sterile conditions 2 ml of blood was withdrawn from antecubital vein of forearm. The blood sample was then centrifuged at 3000 rpm to obtain serum.

The saliva collected was unstimulated saliva. All volunteers were asked to thoroughly wash their mouth before collection of saliva. The patient was seated comfortably and the saliva was collected at every 30 seconds for 5 minutes in sterile container. The saliva sample was then centrifuged at 3000 rpm and supernatant obtained.

SERUM AND SALIVARY GLUCOSE DETERMINATION

Estimation of serum and salivary glucose was done by GOD/POD (Glucose oxidase/Peroxidase) method. After centrifugation 10 µl of serum from blood sample and 10 µl of supernatant from saliva sample were mixed separately with 1000 µl of enzyme reagent and incubated at 37 ºC for 15 minutes. The absorbance of Test (T), Standard (S) and against Blank (B) on photocolorimeter with green filter (540 nm) was measured.

Calculations

$$\text{Glucose in mg\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 100 \text{ (Standard concentration)}$$

Statistical Analyses used: The readings of serum and salivary glucose were analysed using non-parametric tests, Mann-Whitney test using Pearson correlation and ROC curve.

Dr Sonam Bhalla, M.D.S, Senior Lecturer, Oral Maxillofacial Pathology & Microbiology, YIDSR, Gadholi, Yamunanagar, Haryana
Tel: +91-8222848088
Email: sonam.c811@gmail.com:

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RESULTS

The total study sample of 500 individuals was divided into two study groups depending on their serum glucose levels. Group 1 included 429 individuals who showed serum glucose levels within the normal range i.e were healthy individuals. Group 2 included 71 individuals who showed raised serum glucose levels i.e were diabetics.

In healthy individuals the serum glucose levels ranged from 48.0 to 160.0 mg/dL, with a mean of 110.43 mg/dL and standard deviation of 17.14. The salivary glucose levels in the healthy individuals ranged from 2.0 to 42.50 mg/dL, with a mean of 10.89 mg/dL and standard deviation of 6.45. When results for normal serum and salivary glucose levels were compared using Pearson correlation, the value for Pearson correlation was 0.311 and the correlation was highly significant ($p < 0.0001$).

In diabetic individuals individuals the serum glucose levels ranged from 152.0 to 354.0 mg/dL, with a mean of 201.33 mg/dl and standard deviation of 49.66. The salivary glucose levels in diabetic individuals ranged from 4.0 to 46.0 mg/dl, with a mean of 18.33 mg/dl and standard deviation of 10.12. When results for serum and salivary glucose in diabetics were compared using Pearson correlation, the value for Pearson correlation was .422 and a highly significant correlation was seen ($p < 0.0001$). Also when results for total serum glucose and salivary glucose were compared, the value for Pearson correlation was 0.469 and correlation was highly significant ($p < 0.0001$). Tests for specificity and sensitivity showed that in the present study a value of 11.9 mg/dl showed 70% sensitivity and 68% specificity.

Table I: Relation between serum glucose concentration and salivary glucose concentration in healthy individuals and diabetics

No. of Patients		Minimum		Maximum		Mean ±S.D*	
	N	Serum glucose (mg/dl)	Salivary glucose (mg/dl)	Serum glucose (mg/dl)	Salivary glucose (mg/dl)	Serum glucose (mg/dl)	Salivary glucose (mg/dl)
Normal	429	48.0	160.0	42.50	110.44± 17.15	10.89 ± 6.46	
RAISED	71	152.0	4.0	354.0	46.0	201.34 ± 49.66	18.33± 10.13
Total	500	48.0	2.0	354.0	46.0	123.34± 40.08	11.95± 7.54

*Standard deviation

DISCUSSION

The present study was undertaken with the aim of suggesting saliva as the diagnostic fluid especially in the diabetics. As this study was conducted to observe the changes in salivary glucose of diabetics in general population, there was no age limit, no exclusion and inclusion criteria and only random blood glucose and salivary glucose samples were studied.

Diabetes mellitus is a group of metabolic disorders that share the common underlying feature of hyperglycemia. Hyperg-lycemia in diabetes results from defects in insulin secretion, insulin action, or, most commonly, both. Chronic hyperg-lycemia and the attendant metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels. Millions of people are affected worldwide and the number continues to rise steeply even in India. Self-measurement of blood glucose is an integral part of diabetes mellitus therapy. As many as 65% of diabetic people

perform some degree of self-monitoring and approximately 20-30% do so frequently. Most patients consider this the most onerous part of their diabetes therapy. It requires obtaining blood and is usually the most painful part of therapy. Patients therefore are anxious for a less-invasive method for glucose measurement.[4]

Oral fluid or whole saliva is a complex chemical milieu of teeth and oral soft tissues, consisting mainly of water, essential electrolytes, glycoproteins, antimicrobial enzymes and numerous other important constituents like glucose and amylase. It may also contain fluid from the gingival pocket (gingival, or crevicular fluid). The increased salivary glucose evident in the whole saliva can be due to several contributing factors or it could be a simple reflection of the blood glucose levels since saliva is an ultra filtrate of plasma. The salivary analytes are derived from plasma generally by three mechanisms (passive diffusion, active transport and ultra filtration) and are thus found in saliva. Jurysta C et al considered salivary glucose to be often as being from glandular origin and located glucose transporters at the baso-lateral membrane and at apical level of acinar cells in parotid gland. Thus glucose transports into the acinar cells and thereafter into the acinar lumen.[5]

Diabetes mellitus has been consistently documented to be associated with altered salivary composition and function. Basement membrane permeability of the parotid gland is reported to be higher in diabetes mellitus, and this results in raised percolation of components such as glucose, amylase and protein from blood, thus raising their levels in saliva.

In the present study using Mann-Whitney test, statistics showed significant ($p < .001$) correlation between serum glucose concentration and salivary glucose concentration. This revealed that changes in salivary glucose followed the changes in serum glucose levels. This was supported by Reuterving CO, Reuterving G, Hägg E and Ericson T (1987) who conducted salivary investigations of diabetics one to five months apart, during different periods of metabolic control. They found that salivary glucose concentration was lower during the period of better metabolic control and concluded that degree of metabolic control affected salivary glucose concentration.[6] In 1991, Darwazeh and coworkers

in another study assayed the glucose concentration in unstimulated mixed saliva and serum and found that in diabetic patients, salivary glucose concentration was significantly higher than in the controls and was directly related to blood glucose concentration.[7]

Borg AA, Birkhed D, Berntorp K, Lindgärde F and Matsson L (1998) in another study found that the concentration of glucose in parotid saliva elevated significantly two hours after glucose or food intake in individuals with diabetes mellitus as compared to healthy individuals.[8] In similar studies Aydin S (2007) and Jurysta C et al (2009) confirmed that the glucose concentration in saliva is higher in diabetics than in healthy individuals.[9,10] This was further supported by Priya SS, Bharani GO, Nagalingam M, Jayanthi M, Kanagavalli U (2011) who in their study concluded that salivary samples of the non-diabetic individuals did not show the presence of glucose in higher concentrations, while the samples obtained from the diabetics showed significant concentrations of glucose in the saliva along with their serum glucose concentration and the salivary glucose concentration was found to correlate with the serum glucose concentration.[11] Another study done by Panda A, Venkatapathy R, Nirima O (2012) showed that fasting salivary glucose levels increased in diabetics as compared to healthy subjects.[12] Similar results were seen in a study done Naik VV, S Yasmin, Pilli GS, Mishra MN (2011) who observed that glucose was detected in the saliva of both diabetic and non-diabetics. In their study the postprandial salivary glucose values in the control group ranged from 12.5 to 20.0 mg/dl and in diabetics ranged from 15.3 to 30.7 mg/dl. Thus they observed that as blood glucose levels changed in post-prandial samples, so did salivary glucose levels, irrespective of age and sex.[13]

Using ROC curve for sensitivity and specificity in the present study a value of 11.9 mg/dl showed 70% sensitivity and 68% specificity. Thus 70% of individuals with salivary glucose levels > 11.9 mg/dl were true positives i.e diabetics and 68% of individuals with salivary glucose levels < 11.9 mg/dl were true negatives i.e non-diabetics. The average salivary glucose in non-diabetics in the present study was 10.8973 mg/dl and that in the diabetics was 18.3306 mg/dl. The average salivary glucose in non-diabetics is considerably higher than that seen in study done by Soares MSM, Batista Filho MMV, Pimentel MJ, Passos IA, Chimenos KE (2009) who found the average salivary glucose concentration in healthy adults to be 5.94 mg/dl.[14] This could be because the saliva sample in the present study was not from the fasting individuals, rather was random and saliva glucose could have been affected by the last time of carbohydrate intake. Moreover few factors such as oral retention of alimentary carbohydrates, glucose utilization by oral bacteria, release of carbohydrates from salivary glycoproteins and contamination of saliva by a large outflow of crevicular fluid in patients with a poor gingival status may also alter the level of salivary glucose.

Amer S, Yousuf M, Siddiqui PQ, Alam J (2001) in their study observed that glucose could only be detected in the salivary samples of subjects with diabetes mellitus, but none of the salivary samples from the non-diabetic control subjects showed the presence of glucose. They also observed that salivary glucose concentration showed a parallel increase with an increase in the serum glucose concentration.[15] On the other hand Forbat LN, Collins RE, Maskell GK and Sönksen PH (1981)[16] and Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME and Peydró A (2006)[17] revealed that salivary glucose levels did not reflect blood glucose levels and that the structural modifications in the salivary gland in diabetic patients could result in variations in the salivary composition. In yet another study Hegde A, Shenoy R, D'Mello P, Smitha A, Tintu A, Manjrekar P (2010) confirmed that salivary glucose concentrations showed no difference between the diabetics and non- diabetic healthy control group, implying association of high plasma glucose with high salivary glucose levels to be an infrequent observation which may be affected by metabolic control of the disease. They concluded that fasting plasma glucose is a better indicator of glycemic status as it was significantly higher in diabetic group as compared to control group.[18]

Conclusion

It can thus be inferred that glucose may be present in saliva of both healthy and diabetic individuals but glucose concentration in saliva is higher in diabetic patients than in healthy individuals. Moreover the changes in serum glucose concentration are followed by the changes in salivary glucose concentration. The current techniques for blood glucose monitoring are limited by location, equipment, supplies and being invasive and painful. Nonetheless the collection of saliva is convenient, noninvasive and cost-effective. Moreover easy and non painful self monitoring of blood glucose is of tremendous value as a part of diabetes therapy. Thus saliva may be used as an adjunct diagnostic tool in diabetes mellitus.

Nevertheless the present study may set the scene for further investigation and newer studies for estimation and correlation of fasting blood glucose and fasting salivary glucose with larger population samples.

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