

Original Research Article

A non-invasive method to estimate glucose levels in salivary secretions of diabetic patients

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ABSTRACT

Background: Aim of the study is to correlate between blood glucose levels and salivary glucose levels in type 2 diabetic patients, to study the relationship between salivary glucose levels and serum glucose levels in type 2 diabetic patients and to determine whether salivary glucose levels could be used as a non-invasive tool for the measurement of glycemic control in type 2 diabetics. This requirement of multiple pricking at regular intervals for monitoring serum glucose levels in the body is physically and psychologically traumatic to the patient. This necessitates a non-invasive procedure like salivary glucose estimation.

Methods: The study population consisted of two groups: Group 1 consisted of 20 controlled diabetics and Group 2 consisted of 20 diabetics based on their random blood and salivary glucose levels. Two milliliters of peripheral blood were collected for the estimation of random blood glucose levels. Unstimulated saliva was collected by the oral rinse technique for the estimation of salivary glucose.

Results: ANOVA single factor and Pearson correlation coefficient was carried out to know the statistical significance between the two groups. The salivary glucose levels were significantly higher in controlled and when compared with the diabetics. The salivary glucose levels showed a significant correlation with blood glucose levels, suggesting that salivary glucose levels can be used as a monitoring tool for predicting glycemic in diabetic patients.

Conclusions: The present study found that estimation of salivary glucose levels can be used as a non-invasive, painless technique for the measurement of diabetic status of a patient in a dental set up.

Keywords: Glucose, Glycemia and serum, Saliva, Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by absolute deficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues.¹ Diabetes can be divided into two types based on the age group. In Type I beta cells of Langerhans cells are destroyed in pancreas. In type II insulin resistance is developed. Diabetes has variable effects on oral mucosa.² Patients with poor oral hygiene are very prone to oral infections. There are so many

ongoing researches in past decades on various body fluids in comparison with blood for diagnosing purpose. One of the most important and abundant secretions in the human body is saliva.³ This clear fluid that is usually taken for granted has many magical qualities when associated with health of the body. Hence salivary glucose levels can be used as a non-invasive indicator of blood glucose levels. Hence, the present study was being undertaken to detect the salivary glucose levels in controlled diabetic and uncontrolled diabetic patients.

METHODS

All known type II diabetic patients visiting the outpatient department of the Government Medical College and Hospital were included in the study. This study was conducted for 6 months during the year (January 2017 to June 2017). The study population consisted of two groups. Group 1 consisted of 20 controlled diabetic individuals whose random non fasting plasma glucose levels were in the range of 130-200 mg/dL. Group 2 consisted of 20 diabetics with random non fasting plasma glucose levels greater than 200-450 mg/dL with uncontrolled diabetics. Blood glucose estimation was done by collecting blood. All blood samples were collected after breakfast. Patient was asked to sit on chair and explained the purpose of taking their blood, 2ml of venous blood was collected. Serum glucose levels were measured using the glucose oxidase method in a semiautomated analyzer. The serum samples (10 µL) were mixed with the (1000 µL) reagent (glucose oxidase) and incubated for 5 min at 37°C. The absorbance values of the standard and the sample against the reagent were measured. Saliva was collected for glucose estimation. All salivary samples were collected after breakfast. Unstimulated saliva was collected using the "spit technique." The patient was asked to sit in the dental chair with the head tilted forward and instructed not to swallow during the procedure or swallow any saliva if present in the mouth. Then, the patient was instructed to spit in a sterile graduated container every minute for 10 min.

Salivary glucose levels were measured using the glucose oxidase method in a semiautomated analyzer. The saliva sample (10 µL) was mixed with the (1000 µL) reagent

(glucose oxidase) and incubated for 5 min at 37°C. The absorbance values of the standard and the sample against the reagent black were measured. The glucose standard was diluted 10 times for estimation of salivary glucose. This method was standardized and could measure a minimal salivary glucose concentration of 0.5 mg/dL.

RESULTS

Analysis of Variance (ANOVA) has been used to find the significance of the study parameters between two groups of patients, and multiple other comparisons were made between two groups. The Pearson correlation (r) coefficient was performed.

In this study, when the distribution of random non fasting plasma glucose levels in the two groups were studied, the blood glucose levels varied between in controlled diabetics is 130-200 mg/dL and in controlled individuals 200-450 mg/dL. The salivary glucose levels among the two groups varied between 4.2-8.8 in controlled diabetics and 11.3-16.2 mg/dL mg/dL in uncontrolled diabetic individuals. In the controlled diabetic group, the sera glucose levels ranged from 130 to 200 mg/dl with a mean of 152.3 mg/dl and a SD of 17.7 against salivary glucose levels that ranged from 4.2 to 8.8 mg/dl with a mean of 6.555 mg/dl and a SD of 1.58. In the uncontrolled diabetic group, the sera glucose levels ranged from 200 to 450 mg/dl with a mean of 328.7.6 mg/dl and with a SD of 70.3, whereas the salivary glucose levels mean value is 13.83 mg/dl and a SD of 1.49. Further, there was a positive correlation between fasting serum glucose levels and salivary glucose levels found when Pearson correlation coefficient was done.

Table 1: Controlled and uncontrolled diabetic group between salivary glucose levels.

ANOVA: Single factor					
Summary					
Groups	Count	Sum	Average	Variance	
controlled diabetic group	20	131.1	6.555	2.508921	
controlled diabetic group	20	3358.8	167.94	532.3541	
ANOVA					
Source of variation	SS	df	MS	F	p-value
Between groups	260451.2	1	260451.2	973.8986	p<0.001
Within groups	10162.4	38	267.4315		
Total	270613.6	39			

Statistical analysis: ANOVA one-way test. Statistically significant if p<0.05

Table 2: Pearson correlation coefficient between serum and salivary glucose levels of controlled group.

	Serum glucose value	Salivary glucose value
Serum glucose value Karl Pearson's correlation coefficient value (r)	1	0.441
p		0.025
Salivary glucose value Karl Pearson's correlation coefficient value (r)	0.441	1
p		0.025

Table 3: Pearson correlation coefficient between serum and salivary glucose levels of uncontrolled diabetics.

	Serum glucose value	Salivary glucose value
Serum glucose value Karl Pearson's correlation coefficient value (r)	1	0.458
p 0.021		
Salivary glucose value Karl Pearson's correlation coefficient value (r)	0.458	1
p 0.021		

Table 4: Controlled and uncontrolled diabetic group between serum glucose levels.

ANOVA: Single Factor					
Summary					
Groups	Count	Sum	Average	Variance	
controlled diabetic group	20	3358.8	167.94	532.3541	
uncontrolled serum	20	6748.63	337.4315	4060.1	
ANOVA					
Source of variation	SS	Df	MS	F	p-value
Between groups	287273.7	1	287273.7	125.1068	p<0.001
Within groups	87256.62	38	2296.227		
Total	374530.3	39			

Statistical analysis: ANOVA one-way test. Statistically significant if p<0.05.

The difference in salivary glucose levels between the two groups was calculated using ANOVA one-way test and was found to be statistically significant (p<0.001) (Table 1).

The correlation coefficient between sera and salivary glucose levels, in this group, gave as p value is 0.025 which was found to be statistically significant, r value being 0.441 and p value is 0.025 (Table 2). The correlation coefficient between sera and salivary glucose levels of uncontrolled diabetics were again statistically significant p value is 0.021. In the controlled diabetic group and uncontrolled group r value is 0.458 and p value is 0.021 (Table 3) respectively.

The difference in serum glucose levels between the two groups was calculated using ANOVA one-way test and was found to be statistically significant (p<0.001) (Table 4).

DISCUSSION

Results shown were there was increase in the salivary glucose levels in uncontrolled diabetics as compared with control patients. These findings are similar to the findings of Andersson et al, who observed that salivary glucose levels were raised in diabetic subjects when compared with nondiabetic subjects after oral glucose loading.⁴ The salivary samples of the nondiabetic control subjects did not show the presence of glucose even in the slight concentrations, while the samples obtained from the diabetics showed significant change.

This finding suggests that salivary glucose levels likely follow a threshold mechanism. The increase in salivary glucose levels with increase in blood glucose levels has

been suggested to be "leakage" across the basement membrane of the glands, particularly the parotid gland, when blood glucose levels increase beyond a threshold value.⁵ Therefore, it can be said that in nondiabetics, because of the absence of disease process, there is no change in the basement membrane permeability leading to less amounts of glucose in saliva as seen in this study. Epidemiological studies in India have shown high incidence of Diabetic Mellitus (DM) in the year 2002. It was estimated that there were 19.4 million individuals affected by type II DM, by year 2025 which is likely to increase up to 57.2 million. Routine blood examination is a painful procedure and cause trauma to the patient, and hence an alternative procedure is being explored, among which salivodiagnosis is most promising. Saliva as a diagnostic tool is not limited to oral diseases but have been extended to the entire physiologic system, as most components found in the blood are also found in the saliva. Accordingly, saliva can reflect the physiologic state of the body which includes emotional, endocrinal, nutritional, and metabolic variations, and acts as a source for monitoring oral and systemic health. In previously published studies it was proven that salivary glucose estimation has increased values in type II diabetic mellitus.⁶

According to previous studies, found significant differences (p<0.05) in the buffering capacity between type II 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. This study proved to be the same study reported by Collin et al.⁷

Demmer et al, reported that, in patients with type II DM, the risk of periodontal disease is three times higher than that in the general population. Similarly, it was found that the type II DM patients had significantly poor periodontal status than the healthy controls. This is in accordance to previous studies. 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.⁸

Panchbhai et al, observed significantly elevated mean salivary glucose levels in both uncontrolled and controlled diabetic patients when compared with the healthy controls in accordance with the results of their study.⁹

According to the parameters, uncontrolled random blood glucose levels between salivary and serum showed significant positive correlation ($p < 0.001$). controlled diabetics showed significant correlation ($p < 0.001$). By this it was known that there is a positive correlation between blood and salivary glucose levels. Hence salivary glucose levels can also be used as a diagnostic tool in monitoring type II diabetics.¹⁰ Type II DM is known to affect both the sympathetic and parasympathetic nervous system of the salivary glands, which results in decreased salivary secretion, microangiopathy, dehydration, and hormonal changes, which may contribute to the decrease in the salivary flow rate.

CONCLUSION

On the basis of the present study it is concluded that saliva contains glucose which has varied proportions with that of serum glucose levels and their correlation between the saliva and serum was significant. Sailodiagnosis can be used as an alternative to serum to find the glucose levels. This method is noninvasive and cost effective for screening in large population. Instead of all this to prevent diabetics, do regular exercise, be on diet and decrease obesity, by preventing the morbidity and mortality associated with this dreadful and complex metabolic disorder which seems to be attacking people in all age groups, genders and with varied socioeconomic status.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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