Estimation Of Salivary Glucose Level And Plasma Glucose Level In Subjects With And Without Diabetes Mellitus: A Comparative Study.

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Abstract: Objective: The objective of this study was to estimate and correlate salivary glucose levels and plasma glucose levels in non-diabetic subjects, controlled and uncontrolled diabetic subjects and to assess if salivary glucose can be a potentially useful non-invasive tool in diagnosing diabetes mellitus and in monitoring of glycemic control in diabetic patients. **Materials and methods:** A total of 90 subjects aged between 40-60 years participated in the study. Diabetic status was determined by estimation of random non-fasting plasma glucose levels and Glycosylated haemoglobin levels. Both unstimulated and stimulated saliva were collected and investigated for glucose levels. Salivary glucose levels were measured using the glucose oxidase method. **Results:** Salivary glucose levels were significantly higher in diabetics than non-diabetics. Mean un-stimulated salivary glucose level was 1.15 mg/dL in control group, 2.04 mg/dL in controlled diabetic group and 3.99 mg/dL in un-controlled diabetic group. There was a significant positive correlation between salivary and plasma glucose levels. **Conclusion:** These results show that salivary glucose concentration can be used as a potentially useful non-invasive tool for diagnosing diabetes mellitus and monitoring glycemic control in diabetic patients. [Jha S NJIRM 2014; 5(3) :65-70]

Key Words: Diabetes mellitus, Salivary glucose, Serum glucose

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Introduction: Diabetes mellitus is a complex multi-system disorder characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues.[1] It is the most common endocrine disorder with potentially devastating complications that affects all age groups worldwide.

Data indicates that in 2011, 366 million people worldwide were affected by diabetes and the number is continuing to climb steeply. By 2030, predictions are that the number of people with diabetes will reach 552 million. Currently India is in the second position after the People's Republic of China. [2]

Type 2 diabetes mellitus is described as a global epidemic fuelled by population growth, ageing, urbanisation, increasing obesity, and changing lifestyles.

The impact of diabetes is felt in both developed and developing countries, because of the high morbidity and mortality associated with its complications in renal, retinal, nervous and vascular system. [3]

Diabetes has variable, and sometimes profound, effects on the oral tissues. Patients with poor glycemic control are particularly prone to severe and/or recurrent oral infections. [3]

About one third of people with diabetes are undiagnosed. The average lag between the actual onset and the diagnosis of type 2 diabetes is 7 years. [4] This delay in diagnosis is the main reason for the profound complications associated with diabetes. So, early diagnosis of diabetic condition can prevent the complications and related morbidity and mortality.

Currently, a diagnosis of diabetes is achieved by evaluating blood glucose levels. Monitoring blood glucose at frequent intervals causes unnecessary discomfort and mental trauma to patients; therefore, a much simpler and non-invasive technique for the diagnosis and monitoring of diabetes is very desirable.

The present study was conducted to determine the role of saliva as a diagnostic tool by estimating and correlating serum glucose and salivary glucose in the diabetic and non-diabetic groups.

Materials And Methods:

1. **Collection of Samples:** All the known diabetic patients and non-diabetic subjects were selected from department of Oral Medicine and Radiology; Dayananda Sagar College of Dental Sciences, Bangalore. The written consent of the patients was obtained for saliva and blood collection and the procedures were explained to them. The ethical clearance was obtained from IRB.
2. **Diagnostic criteria for diabetes:** The subjects were divided into 3 groups based on their random blood glucose. Group I: non-diabetic subjects (n = 30) composed of patients 40-60 years old who had random non-fasting plasma glucose (RNFPG) values 80-120 mg/dL; group II (controlled diabetic subjects(n =30) composed of patients 40-60 years old who had RNFPG values >120<200 mg/dL; and group III (uncontrolled diabetic subjects; n =30) with RNFPG >200 mg/dL.
3. **Serum glucose determination:** Subsequent to a written consent, 2 ml of peripheral venous blood was collected and RNFPG levels were measured using glucose-oxidase method and Glycosylated haemoglobin (HbA1c) levels were measured using the ion exchange resin method.
4. **Saliva collection and Measurement of salivary glucose levels:** All salivary samples were collected 2 hours after breakfast. Unstimulated and stimulated saliva was collected using a "suction technique." For unstimulated saliva collection the patient was asked to sit in the dental chair with head tilted forward and instructed not to speak, swallow, or do any head movements during the procedure, or swallow any saliva if present in the mouth. Then the accumulated saliva was collected using suction method. Stimulated saliva was collected using 2% food-grade citric acid that was applied to the dorsolateral surface and the tip of the tongue every 30 seconds, and the pooled saliva was collected using suction method into a sterile container. Salivary samples thus collected represented whole mouth fluid contributed by secretions from major and minor salivary glands and potentially gingival crevicular fluid.

   Glucose levels of stimulated and unstimulated saliva were measured using the glucose oxidase method in a semiautomated analyzer. The saliva sample (100 uL) was mixed with the reagent in a 1:3 ratio and incubated for 5 minutes at 37°C. The absorbance values of standard and the sample against the reagent blank was measured. The glucose standard was diluted 10 times for estimation of salivary glucose levels. The method was standardized and could measure a minimal slivary glucose concentration of 0.2 mg/dL.
5. **Statistical analysis :**All statistical analysis were performed with the Statistical Package for the Social Sciences (SPSS version 11) software. The differences in mean between groups were assessed using ANOVA and the multiple comparison between different parameters was done using Bonferroni test.

Results: The study consisted of 90 patients, divided into three groups: control group, controlled diabetic group and un-controlled diabetic group each consisting of 30 patients.

In the control group, the mean serum glucose level was 103.30 mg/dL, mean unstimulated salivary glucose level was 1.15 mg/dL and the mean stimulated salivary glucose level was 0.98 mg/dL. The correlation coefficient between serum glucose and unstimulated and stimulated salivary glucose was calculated and the *r* value was found to be 0.663, and 0.512 respectively, which was statistically significant ($P < 0.001$).

In the controlled diabetic group, the mean serum glucose level was 169.23 mg/dL, the mean unstimulated salivary glucose level was 2.04 mg/dL and the mean stimulated salivary glucose level was 1.88 mg/dL. The correlation coefficient between serum glucose and unstimulated and stimulated salivary glucose was calculated and the *r* value was

found to be 0.0.847, and 0.830 respectively, which was statistically significant ($P < 0.001$).

In the uncontrolled diabetic group, the mean serum glucose level was 290.00 mg/dL, the mean unstimulated salivary glucose was 3.99 mg/dL and mean stimulated salivary glucose level was 3.61 mg/dL. The correlation coefficient between serum glucose and unstimulated and stimulated salivary glucose was calculated and the *r* value was found to be 0.704, and 0.636 respectively, which was statistically significant ($P < 0.001$).

Table 1: Correlation between different parameters (RNFPG, HbA1c, USSG, SSG) in control group.

Control Group		Random Plasma Glucose	HbA1c	Unstimulated Salivary Glucose	Stimulated Salivary Glucose
Random Plasma Glucose	r	1	0.315	0.663	0.512
	P-Value	---	0.090	<0.001*	0.004*
HbA1c	r	0.315	1	0.312	0.400
	P-Value	0.090	---	0.094	0.029*
Unstimulated Salivary Glucose	r	0.663	0.312	1	0.857
	P-Value	<0.001*	0.094	---	<0.001*
Stimulated Salivary Glucose	r	0.512	0.400	0.857	1
	P-Value	0.004*	0.029*	<0.001*	---

Correlation between random plasma glucose, unstimulated salivary glucose and stimulated salivary glucose was calculated in three groups.

We found that the salivary glucose levels increased as serum glucose levels increased with the correlation coefficient between serum glucose and unstimulated and stimulated salivary glucose in control subjects being 0.663, and 0.512 respectively, in controlled diabetics being 0.847 and 0.830 respectively and in uncontrolled diabetics being 0.704 and 0.636 respectively. These correlations were statistically significant with ($P < 0.001$).

Similarly we measured HbA1c levels in the three groups and found that the mean HbA1c level in control group was 5.30%, in controlled diabetic group was 7.24%, in uncontrolled diabetic group was 10.14%, with a significant positive correlation brtween each group. The probability value denoted significant correlation between both HbA1c and stimulated salivary glucose and HbA1c and unstimulated salivary glucose (p<0.001).

Graph 1: Scatter plot graph showing correlation between different parameters (RNFPG, HbA1c, USSG, SSG) in control group.

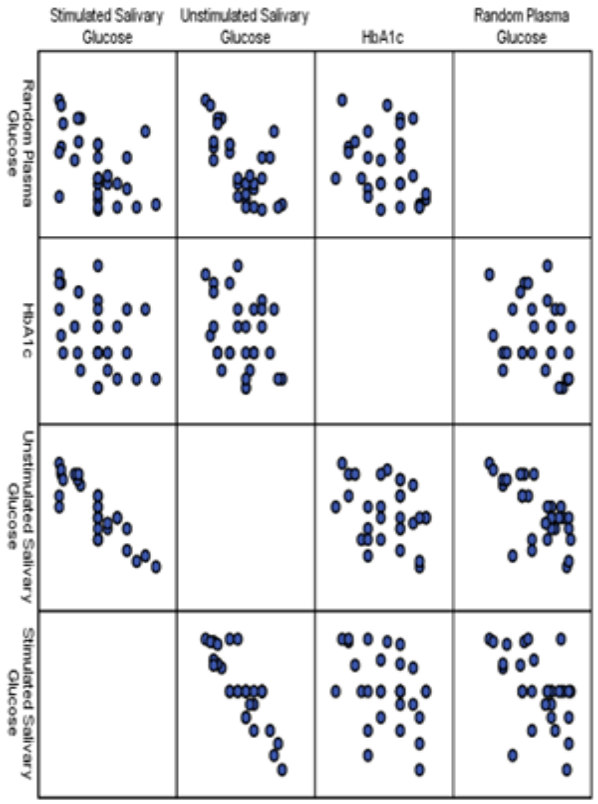


Table 2: Correlation between different parameters (RNFPG, HbA1c, USSG, SSG) in controlled diabetic group.

Controlled Diabetic Group		Random Plasma Glucose	HbA1c	Unstimulated Salivary Glucose	Stimulated Salivary Glucose
Random Plasma Glucose	R	1	0.681	0.847	0.830
	P-Value	---	<0.001*	<0.001*	<0.001*
HbA1c	R	0.681	1	0.700	0.646
	P-Value	<0.001*	---	<0.001*	<0.001*
Unstimulated Salivary Glucose	R	0.847	0.700	1	0.956
	P-Value	<0.001*	<0.001*	---	<0.001*
Stimulated Salivary Glucose	R	0.830	0.646	0.956	1
	P-Value	<0.001*	<0.001*	<0.001*	---

Discussion: Our study was aimed to estimate plasma glucose level and salivary glucose level, to correlate plasma and salivary glucose level, and to assess if salivary glucose level can be used as a non-invasive means of diagnosing and monitoring diabetes mellitus.

We found that the salivary glucose levels increased as serum glucose levels increased with the

correlation coefficient between serum glucose and unstimulated and stimulated salivary glucose in control subjects being 0.663, and 0.512 respectively, in controlled diabetics being 0.847 and 0.830 respectively and in uncontrolled diabetics being 0.704 and 0.636 respectively. These correlations were statistically significant as the probability values (*p* value) in all the three groups were below 0.001.

Graph 2: Scatter Plot Graph Showing Correlation Between Different Parameters (RNFPG, Hba1c, USSG, SSG) In Controlled Diabetic Group.

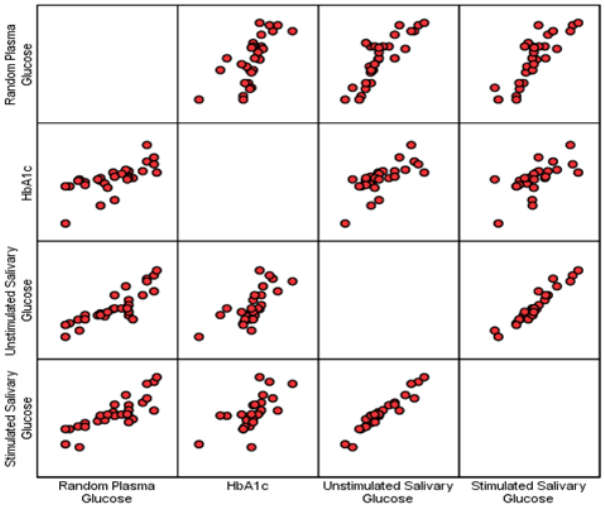
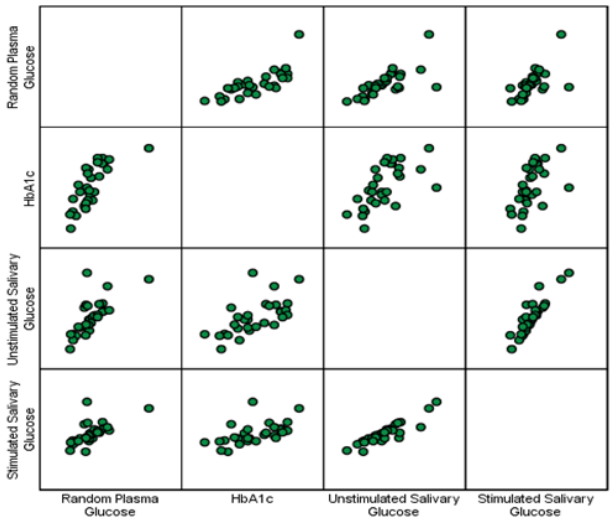


Table 3: Correlation Between Different Parameters (Rnfpg, Hba1c, Ussg, Ssg) In Un-Controlled Diabetic Group.

Uncontrolled Diabetic Group		Random Plasma Glucose	HbA1c	Unstimulated Salivary Glucose	Stimulated Salivary Glucose
Random Plasma Glucose	r	1	0.770	0.704	0.636
	P-Value	---	<0.001*	<0.001*	<0.001*
HbA1c	r	0.770	1	0.634	0.569
	P-Value	<0.001*	---	<0.001*	0.001*
Unstimulated Salivary Glucose	r	0.704	0.634	1	0.904
	P-Value	<0.001*	<0.001*	---	<0.001*
Stimulated Salivary Glucose	r	0.636	0.569	0.904	1
	P-Value	<0.001*	0.001*	<0.001*	---

Our result was in accordance with Abikshyeet.P et al [2] who in a study to substantiate the role of saliva as a diagnostic and monitoring tool for diabetes, compared salivary glucose with blood glucose in healthy and diabetic subjects, and found the correlation coefficient between serum glucose and salivary glucose in the control group to be 0.5216, which was statistically significant (*P* , 0.05). The correlation coefficient between serum glucose and salivary glucose in the patient group was 0.7686, which was highly significant (*P* , 0.01).

Graph 3: Scatter Plot Graph Showing Correlation Between Different Parameters (RNFPG, Hba1c, USSG, SSG) In Un-Controlled Diabetic Group.



Shashikumar.R et al [3] in a study on 150 subjects aged between 40-60 years and comprising of three groups; Glucose levels of stimulated and unstimulated saliva were measured using the glucose oxidase method in a semi-automated analyzer (similar to our study). They found salivary glucose levels to be significantly higher in diabetic subjects than in non-diabetic subjects.

Aydin.S [5], on 62 subjects measured glucose using glucose-oxidase method, and statistical significance of differences in glucose between study group and controls was estimated by analysis of variance (ANOVA). They found salivary glucose to be significantly higher in obese as well as non-obese diabetic subjects than in controls (p<0.05).

Jyrusta.C et al [6] used the similar method (hexokinase method) in their study to estimate salivary glucose and found that salivary glucose concentration was much higher in diabetic patients than in control subjects, in both unstimulated and stimulated saliva.

Panchbhai A.S et al [7] on a study on 120 subjects estimated salivary glucose using glucose oxidase method and found that mean salivary glucose levels were higher in uncontrolled and controlled

diabetic groups than in the healthy non-diabetic group and the differences were highly significant.
Amer.S et al [8] found a positive correlation of (r = 0.78) between salivary and serum glucose concentration among the diabetics using enzymatic colorimetric test kit, GOD-PAP (Plasmatec, U.K.). In our study we found a positive correlation of 0.847 between serum glucose and unstimulated salivary glucose and 0.830 between serum glucose and stimulated salivary glucose. This could be because of difference in age group taken (35-45 years in their study) and (40-60 years in our study); difference in methodology (GOD-PAP in their study and GOD-POD in our study) and because of improvised technique used.

Forbat et al [9] concluded that salivary glucose levels did not reflect blood glucose levels. Although they used the similar method (glucose oxidase method) to estimate salivary glucose, the negative result could be because they used pure samples of parotid fluid rather than whole saliva as in our study.

On comparision of serum glucose and salivary glucose in diabetic patients done by Sreedevi et al [10], they found a highly significant correlation between salivary glucose and serum glucose before the treatment and after the control of diabetes.

Carmen Carda et al [11] found that salivary glucose was augmented in patients with poor metabolic control.

In a study done by Ana Carolina et al [12] on comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients they found that salivary glucose concentration was significantly higher in the diabetic patients than in non-diabetic individuals. 2010

CONCLUSION: On the basis of our study we can conclude that saliva contains glucose which varies in proportionate to its serum concentration, and this correlation between salivary and serum glucose is statistically significant.

Thus saliva offers an alternative to serum as a biologic fluid that can be analysed for diagnosing and monitoring diabetes mellitus.

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