

## A correlative study of salivary and plasma glucose levels in type 2 diabetic patients with and without complications

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### Abstract

**Introduction:** There are contradictory reports on using salivary glucose in diagnosis of diabetes mellitus. This study has made an attempt to estimate the salivary glucose levels in diabetic patients with and without complications and correlate them with plasma glucose as a marker of type 2 diabetes mellitus.

**Method:** This was a Cross sectional observational study. Thirty eight type 2 diabetic patients without complications and thirty eight type 2 diabetic patients with either microvascular or macrovascular complications were the subjects of this study. Age and sex-matched normal healthy individuals were included as controls. Plasma and salivary glucose levels were estimated by spectrophotometric method.

**Results:** We observed significantly higher glucose levels in plasma and saliva, in diabetic patients with complications, in comparison to those without complications, and normal healthy controls. There was significant positive correlation between salivary glucose and plasma glucose among all the subjects of the study.

Non-linear regression analysis shows exponential relationship between plasma and salivary glucose.

**Conclusion:** Salivary glucose analysis offers noninvasive, sensitive method for assessment of severity of diabetic complications with suitability for repeated sampling. Further studies with larger sample size need to be taken up to establish salivary glucose as a marker of diabetes mellitus.

**Key words:** Plasma glucose, Salivary glucose, Type 2 diabetes mellitus.

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### Introduction

Diabetes Mellitus is a clinical syndrome characterized by hyperglycemia caused by absolute or relative deficiency of insulin. It is classified into type 1 and type 2 based on etiologies. Type 1 diabetes mellitus is as a result of the antibodies, destroying the beta cells of the pancreas and type 2 diabetes mellitus is because of resistance of peripheral tissues to insulin<sup>(1)</sup>. At present India is the world leader in the number of people with diabetes (51 million) and there is expected to be an increase in this number to 87 million by 2030, and this will account for one-fifth of the world's population of diabetes. Many of the individuals with diabetes go unrecognized which could be due to various factors ranging from cost to compliance to the screening procedures.

Patients with long standing diabetes mellitus develop various microvascular and macrovascular complications. The microvascular complications include retinopathy, peripheral neuropathy, and

nephropathy. Among the macrovascular complications there are atherosclerotic changes, stroke and peripheral vascular disease.

For the diagnosis and prognosis of diabetes various invasive methods are currently practiced which have to be done frequently and can cause discomfort and affect the quality of life of these patients. As a result there is a need to come up with non-invasive procedures with which the patients are compliant<sup>(2)</sup>.

Human saliva reflects the body's health and can indicate local and systemic alterations such that the components of saliva can be related to the metabolic state of the individual. It can be collected non-invasively and does not require specialized skills and can be used for large scale screening program<sup>(1,3)</sup>. Studies have shown that diabetes influences the concentration of glucose in saliva<sup>(1)</sup>. Various studies conducted have shown higher levels of salivary glucose in diabetics as compared to non-diabetics<sup>(4-8)</sup>. Salivary glucose can be an important marker in the diagnosis and glycemic control in diabetic patients<sup>(9)</sup>.

Use of saliva as a marker being non-invasive, stress free, can be easily acquired from diabetics and hence has advantages. Among the advantages are its easy collection and preservation. This thought has served as a foundation for many studies on salivary glucose levels in diabetics.

However the role of this noninvasive technique in the diagnosis of Diabetes remains controversial as studies have also shown no significant difference in the

glucose concentration in the saliva of diabetics and healthy individuals<sup>(10)</sup>.

There are varied reports of salivary glucose in the previous studies done on Type II DM population and there are limited studies evaluating the salivary glucose levels in complicated cases of Type II DM. To study the effects of increased plasma glucose on salivary glucose levels, we have aimed to estimate glucose levels in plasma and saliva in type 2 diabetic patients with complications in comparison to type 2 diabetic patients without any complications, and to correlate salivary glucose level with plasma glucose in diabetic patients.

### Materials and Methods

This cross sectional observational study was done in the Department of Biochemistry at Father Muller Medical College Hospital, Mangalore. Ethical clearance was obtained from Institutional Ethics Committee. Study was carried out for a period of 3 months. Voluntary informed consent was taken from all the subjects. The study involved 3 groups:

First group comprised of 38 normal healthy individuals, aged 30-70 years. Second group comprised of 38 patients with Type II diabetes without complications, aged 30-70 years. Third group comprised of patients with Type 2 Diabetes Mellitus patients with either microvascular or macrovascular complications like retinopathy, nephropathy, stroke, coronary artery disease, peripheral vascular disease, cerebrovascular disease; aged 30-70 years. Clinically proven cases of type 2 diabetes mellitus were selected as cases<sup>(11)</sup>.

Type 1 Diabetes Mellitus, Gestational Diabetes Mellitus, Pregnancy, Prior surgery of salivary glands, those undergoing treatment with radiotherapy of head

and neck region, those with Sjogren's syndrome ,rheumatoid arthritis or lupus erythematosus, gout, Chronic smokers, alcoholics and tobacco chewers, Chronic illness – liver diseases, acute or chronic renal failure, non-diabetic nephropathy, infections were excluded from the study.

About 2 ml of venous blood was drawn in EDTA-fluoride vacutainers from subjects (from large peripheral vein) under aseptic precautions, centrifuged and the separated plasma used for estimation of glucose by Colorimetric, Glucose oxidase – peroxidase method.

Unstimulated whole saliva samples was collected. The subjects were asked to rinse the mouth with distilled water thoroughly to remove any food debris and then to spit into a sterile small plastic container. Once the saliva (2 ml) was collected, the plastic container was placed in an ice carrier box and transferred to the laboratory for biochemical analysis. Saliva sample was centrifuged at 3000 rpm for 15 min and the supernatant taken for analysis of glucose levels<sup>(12)</sup>. Estimation of salivary glucose was done by Colorimetric, Glucose oxidase– peroxidase method.

### Statistical analysis

To determine the significance of difference of values among three study groups, Welch ANOVA test was used. For multiple group comparisons, Games Howell post-hoc test was used to determine the significance of differences. Spearman's rho correlation test was used to find the strength of association between variables, and non-linear regression analysis was used to predict the changes of salivary glucose levels in three groups. Statistical analysis was done using SPSS 23 software.

### Results

**Table 1: Difference between healthy normal subjects, Type II DM and Type II DM with complications**

	Healthy Normal subjects (n=38)	Type II DM(n=38)	Type II DM with complications(n=38)	P
	Mean±Standard Error (95% CI)	Mean±Standard Error (95% CI)	Mean ± Standard Error (95% CI)	
Salivary Glucose (mg/dl)	1.06±0.08 (0.89-1.23)	3.12±0.59(1.92-4.37)	4.35±0.54 (3.24-5.46)	<0.001
Plasma Glucose (mg/dl)	87.68±1.91(83.80-1.56)	130.90±9.35 (131.14 - 166.84)	148.99±8.80 (131.14 - 166.84)	<0.001

\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001

**Legend 1:** Welch ANOVA test was done. There is significant difference between type II DM, type II DM with complications and healthy subjects groups with respect to salivary and plasma glucose.

**Table 2: Multiple group comparison between normal subjects vs. type II DM; normal vs. type II DM with complications; type II DM vs. type II DM with complications**

	Normal vs. Type II DM	Normal vs. Type II DM with complications	Type II DM vs. Type II DM with complications
	Mean difference±Standard Error (95% CI)	Mean difference ± Standard Error (95% CI)	Mean difference ±Standard Error (95% CI)
Salivary Glucose (mg/dl)	-2.06*±0.60 (-3.53 - -0.60)	-3.29*±0.55 (-4.64 - -1.94)	-1.22±0 .80 (-3.15 - 0.70)
Plasma Glucose (mg/dl)	-43.22*±9.55 (-66.46 - -19.97)	-61.30*±9.01 (-83.23 - -39.37)	-18.08±12.85 (-48.82 - 12.65)

\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001

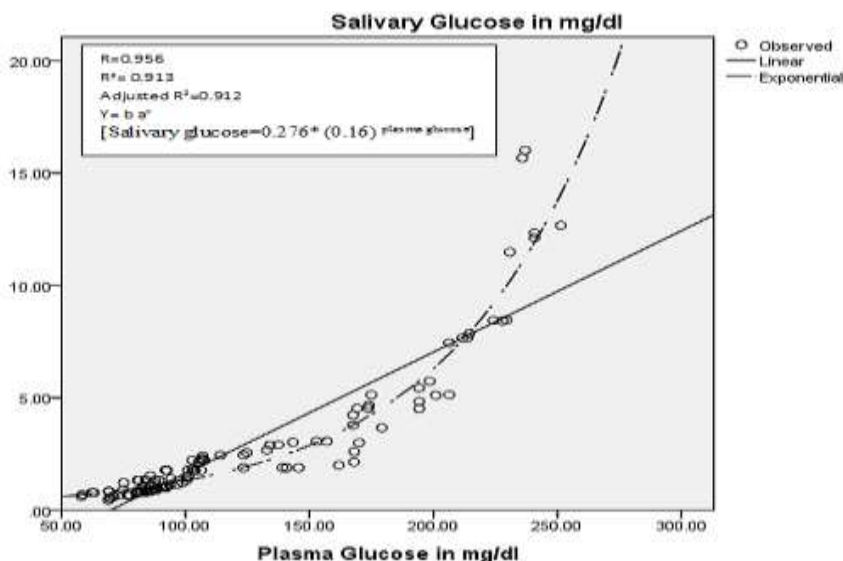
**Legend 2:** multiple group comparison by Games Howell Analysis shows higher levels of salivary glucose & plasma glucose in type II DM & type II DM with complications when compared to normal subjects. There was no significant difference in type II DM when compared to type II DM with complications.

**Table 3: Correlation of salivary glucose and plasma glucose among all the subjects**

Correlations				
			Salivary Glucose in mg/dl	Plasma Glucose in mg/dl
Spearman's rho	Salivary Glucose in mg/dl	Correlation Coefficient	1.000	.958**
		Sig. (2-tailed)	.	.000
		N	114	114
	Plasma Glucose in mg/dl	Correlation Coefficient	.958**	1.000
		Sig. (2-tailed)	.000	.
		N	114	114

\*\* . Correlation is significant at the 0.01 level (2-tailed).

**Legend 3:** Analysis by Spearman's rho test showed that there is significant positive correlation between salivary glucose and plasma glucose among all the study groups. Correlation was done across all the three groups.



**Fig. 1: Exponential Regression Analysis between salivary glucose and plasma glucose levels.**

**Legend 4:** Non-linear regression analysis shows exponential relationship between plasma and salivary glucose.

## Discussion

Saliva being a biological fluid mirrors the body's state of health and disease. It shares many biochemical parameters with serum and has been noticed to have certain favorable factors over that of serum and hence is a useful tool in diagnostics<sup>(13)</sup>. Certain biomarkers in saliva can be used for large scale screening and monitoring of systemic disease<sup>(14)</sup>.

Glucose diffuses from blood vessel membranes and passes through blood into gingival crevicular fluids (GCF) and salivary ducts to finally reach the oral cavity. Several studies have shown that, saliva which is the product of ultra-filtration of blood across the salivary gland reflects the components of the blood in proportion<sup>(15)</sup>.

In the present study, there is significant difference between normal vs. type II DM groups, normal vs. type II DM with complications ( $p=0.000$ ). These findings are in accordance with the studies<sup>(5,6,16,17)</sup>. The increased concentration of salivary glucose in poorly controlled diabetics compared to well-controlled diabetics and healthy subjects is attributed to dehydration caused by osmotic diuresis in glucosuria<sup>(18)</sup>.

In our study there is no significant difference of salivary and plasma glucose between type II DM vs. type II DM with complications which may be because of the confounding factor of hypoglycemic treatments.

An earlier study showed significantly elevated salivary glucose concentration in both uncontrolled and controlled diabetics compared to controlled healthy subjects. But there was no significant difference between uncontrolled and controlled diabetics. This was proposed to be due to salivary gland changes as a result of poor metabolic control<sup>(8)</sup>. Study done by Jurysta and Panchbhai suggested that the glucose concentration in saliva was higher in diabetic patients than in control subjects but did not show significant difference between type 1 and type 2 diabetes mellitus<sup>(7,19)</sup>.

Exponential increase in salivary glucose observed in the present study could be due to membrane damage in type II DM associated with complications. According to previous studies done, elevated plasma glucose levels will cause flux of glucose from semi-permeable membrane into saliva<sup>(18,20)</sup>. Another study has observed increased parotid salivary glucose, and have attributed to diabetic membranopathy causing alteration of the basement membrane<sup>(21)</sup>. Few studies have estimated salivary glucose and correlated it with the morphological changes in parotid gland in type 2 diabetic patients and observed that longer duration of the disease leads to fatty infiltration and microangiopathies of salivary glands<sup>(22,23)</sup>.

In comparison to many previous studies, our study has shown significant positive correlation between salivary and plasma glucose in across all the subjects groups, which was statistically significant. These findings are in accordance with the studies<sup>(17,24,16,25,26,20)</sup>.

In contrast, some studies have found salivary glucose levels to decrease in people with long standing diabetes. Others have reported either negative correlation or no correlation between saliva and plasma glucose in unstimulated or stimulated saliva. These studies confirm that there is poor link between glycaemia and glucose concentration or excretion in saliva, at least on an individual basis and these differences might be the cause of selection criterion of sampling technique, time of collection, methodology and the duration of diabetes mellitus and the status of metabolic control of disease<sup>(7,19,27)</sup>.

Main limitations of our study are less sample size, random sample collection, duration of diabetes mellitus and including patients on treatment.

## Conclusions

Our study has shown significant positive correlation between salivary glucose concentration and plasma glucose concentration. Salivary glucose analysis offer noninvasive, sensitive methods for assessment of severity of diabetic complications with suitability for repeated sampling. Further studies with larger sample size need to be taken up to establish salivary glucose as a marker of diabetes mellitus.

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