

## ORIGINAL ARTICLE

# Correlation Between Blood Glucose and Salivary Glucose in Type 2 Diabetes Mellitus Patients

Kaushalya Indunil Dharmakeerthi<sup>1</sup>, Malaka Priyashan Ponweera<sup>1</sup>, Evindu Hasanjana Moragoda<sup>1</sup>, Lahiru Sandaruwan Galgamuwa<sup>2</sup>, Kithsiri Jayasekara<sup>1</sup>, Vidumini Kaluarachchi<sup>3</sup>, Uditha Bulugahapitiya<sup>3</sup>

<sup>1</sup> Department of Medical Laboratory Science, General Sir John Kotelawala Defence University, Dehiwala-Mount Lavinia 10390, Sri Lanka

<sup>2</sup> Department of Parasitology, Faculty of Medicine, Sabaragamuwa University of Sri Lanka, Ratnapura, Sri Lanka

<sup>3</sup> Colombo South Teaching Hospital, B229 Hospital Rd, Dehiwala-Mount Lavinia Sri Lanka

## ABSTRACT

**Introduction:** Diabetes Mellitus (DM) is one of the common global health burdens. Measurement of blood glucose level is invasive and can cause many complications. Salivary glucose has been suggested as a suitable alternative for blood in recent years. The aim of this study was to establish the correlation between blood glucose level and salivary glucose level of type 2 diabetic mellitus patients. **Methods:** A cross sectional study was conducted at a diabetic clinic in a teaching hospital in Sri Lanka. Blood samples were collected to analyze fasting blood glucose and HbA1c levels. Unstimulated whole saliva samples were collected to measure salivary glucose level and salivary flow rate. Pearson's correlation was applied to determine the association between salivary glucose, blood glucose and HbA1c levels. **Results:** A total of 120 type 2 diabetes mellitus patients and 31 healthy controls were participated. Salivary glucose level was significantly higher in DM patients than healthy individuals. Fasting blood glucose level was significantly correlated with salivary glucose levels among DM patients ( $r = 0.201$ ,  $p = 0.027$ ). A significant relationship was also observed between HbA1c and salivary glucose levels among DM patients ( $r = 0.288$ ,  $p = 0.031$ ). **Conclusion:** Measuring salivary glucose levels may have potential to be used as an alternative non-invasive procedure to screen, diagnose and monitor the glycemic conditions of the DM patients.

**Keywords:** Blood Glucose, Salivary Glucose, HbA1c, Diabetes Mellitus

## Corresponding Author:

Lahiru Sandaruwan Galgamuwa, M.Phil  
Email: lahiruhs@yahoo.com  
Tel: +940710574688

## INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies and one of the major causes of early morbidity and mortality worldwide (1). It may be asymptomatic or have symptoms like polydipsia, polyuria, weight loss and tiredness. Diabetes arises as a result of autoimmune destruction of beta cells in the pancreas or due to insulin resistance of body tissue. Uncontrolled DM could lead to multi organs dysfunction and potential loss of life (2).

High incidence of type 2 DM has been reported among people in both low- and high-income populations. It can affect people of any age and approximately 175 million people have been diagnosed with DM worldwide (3). Population growth, ageing, unhealthy diets, obesity and sedentary life style were identified factors to increase the prevalence of DM in developing countries (4). Well-

timed diagnosis and management of diet and exercises are important for DM patients (5). In Sri Lanka, diabetes has increased rapidly from a prevalence of 2.5% in 1990 to 8.5% in 2000 (6). In the most comprehensive study carried out on diabetes from 2005-2006 in seven provinces except for the North and the East, the age-sex standardized prevalence of diabetes for Sri Lankans was 10.3% (males 9.8%, females 10.9%). The projected diabetes prevalence for the year 2030 is 13.9% (7).

The fast blood sugar (FBS) level is the most common indicator for estimating DM prevalence and incidence. A single measurement of glucose is not necessarily representative of a patient's glycemic control over a long period of time (8). Therefore, an effective method in monitoring of the long-term glucose control of DM patients is the measurement of glycosylated proteins, mainly HbA1c (glycosylated hemoglobin) and fructose amine (9).

Blood investigations are invasive procedures and could lead to physical and mental discomfort to the patient and anxiety (10). Saliva can be collected from a patient without any physically invasive procedure and minimal

discomfort (11). The composition of saliva is not affected by day to day activities unless the patient is dehydrated or having an active infection of the salivary glands.

Similar studies were conducted in several countries but very few in the South Asian Region (12-16). Therefore, the applicability of salivary glucose level instead of blood should be tested for local settings. According to the experience in the medical laboratory field in Sri Lanka, nearly 5% of the diabetic patients facing difficulties in blood drawing. This study was conducted mainly to check the applicability of alternative methods to measure blood glucose level. Therefore, this study was designed to determine the correlation between blood glucose level and salivary glucose level of diabetic patients in Sri Lanka. The findings of this research will help the Sri Lankan clinical setup by introducing a patient friendly sample collection method and clinical setting for diabetic detection.

## **MATERIALS AND METHODS**

### **Study design and population**

A cross sectional study was conducted from August to November 2017. One hundred and twenty confirmed type 2 DM patients aged between 24-60 years, attended to a diabetic clinic in a teaching hospital in Sri Lanka were selected for the study. Informed consent was obtained from each participant and those who did not consent, above 60 years of age and pregnant women were excluded from the study. Patients with mouth sores, heavy alcoholics and patients with other dental disorders were also excluded from the study.

Further, thirty one healthy individual aged between 24 — 60 years were selected as controls for the study. Individuals undergoing treatments for chronic disorders were excluded from the study.

### **Data Collection**

Ethical clearance (ERC/2017/598) was obtained from the ethical review committee of Colombo South Teaching Hospital to conduct the study. Before the implementation of the study, information sheets were distributed to all participants and written consent forms were obtained from them. Socio demographic data (age, gender, height, weight, BMI, duration of diabetes, family history of diabetes), family history and clinical history of the patients was collected using an interviewer administrated questionnaire. Clinical records were obtained in order to collect more medical information about the patient.

### **Sample collection**

A total of 5 ml of venous blood was collected from 151 individuals (120 DM patients and 31 Healthy individuals). All participants were instructed to follow a fasting period of 8-12 hours prior to blood sample collection for determining levels of serum glucose and HbA1c. Saliva

was collected from subjects in the morning between 8.00 - 11.00 a.m. In the present study, the unstimulated whole saliva was taken to determine salivary glucose level (17). All participants were instructed not to smoke / brush / eat two hours before saliva collection.

Subjects were asked to spit out saliva and the ice-chilled graduated saliva collector was used to collect saliva from subjects. At the beginning, saliva collected for the initial 30 seconds were discarded to minimize microbial contamination. The patients were instructed to wash their mouths with tap water and to spit two or three times, after which they were told to spit the saliva pooled in their mouths for the following 10 minutes into the sterile sample collection container to estimate the salivary flow rate and salivary glucose levels. The volume of the collected saliva sample was measured and recorded. After that, it was transferred into NaF tubes and was stored in ice until further investigations. Fasting Blood Glucose (FBG) and HbA1c levels were measured from the collected blood samples. Fasting blood glucose was measured by an automated biochemistry analyzer (Konelab prime 30i, Finland). The values were recorded, and reports were issued to the patients.

Fasting salivary glucose (FSG) levels and the flow Rate of saliva per minute were measured using collected saliva samples. All the patients were sitting in an upright position with his head inclined forward. Saliva was collected in the floor of the mouth and then flowed out over the lip. Formed saliva was let to drip into the graduate test tube for 15 minutes. The collection of saliva was expressed as milliliters per minutes. Following sample collection, the samples were centrifuged and stored at 2-8 °C. Each unstimulated salivary sample was centrifuged at 5000rpm for 10 minutes and the clear supernatant was used for glucose estimation (18).

### **Sample processing**

In DM patients, FSG analysis was conducted using three different saliva volumes to select the most sensitive method for glucose detection. Salivary glucose 10x was analyzed using 100µl of saliva, salivary glucose 5x was analyzed using 50 µl and salivary glucose Neat was analyzed using 10µl.

Each saliva supernatant was separated into three groups and a total of 10µl, 50µl and 100µl of supernatant was added to three test tubes. Similarly, 10µl, 50µl and 100µl of standards were added into another three test tubes. Each three saliva supernatant and standard samples were mixed with 1000µl of glucose oxidase reagent separately. The mixture was incubated for 20 minutes at 37 °C. The absorbance was measured at 500nm and compared with standards provided with the reagent kit (BioLabs, France).

HbA1c levels were determined on the EDTA samples

by an automated analyzer (Bio-Rad D 10). Values were recorded and the reports were issued to the patients.

### Statistical Analysis

All data were entered into the MS EXCEL work sheet. SPSS version 23.0 was used to analyze the data. Socio demographic data and results of biochemical investigations were descriptively analyzed. The mean values of individual data were compared using independent sample t test and ANOVA test. Pearson test was used to determine the correlation between blood and salivary parameters. Linear regression was applied to determine the relationships between these biochemical parameters. P values less than 0.05 were considered as statistically significant.

### RESULTS

A total of 120 DM patients (mean  $51.5 \pm 7.36$  years) with the age range of 25 — 60 years were participated in this study. Majority of participants were females, in pre-obese condition and suffering from DM more than 9 years. The majority of participants (70 %) had a family history of diabetes (Table I). Mean age of healthy individuals was  $40.29 \pm 11.42$  years with the age range of 23 — 60 years. A total of 20 % (n=19) was reported that they were diagnosed with Diabetes Mellitus during their pregnancy (Gestational Diabetics). The moderate correlation coefficient was shown between blood glucose level and salivary glucose in 10x method ( $r = 0.359$ ,  $p < 0.001$ ). The salivary glucose concentration in DM patients was significantly higher than in healthy individuals ( $p < 0.001$ ) (Table II).

Pearson correlation was calculated for the relationships between each biochemical parameter in diabetes

**Table I: General characteristics of the diabetic mellitus patients**

Variable	Categories	No. of participants	Percentage (%)
Age (years)	20 – 45	29	24.2
	46 – 55	51	42.5
	56 – 60	40	33.3
Gender	Male	25	20.8
	Female	95	79.2
BMI	18.5 - 22.9 (Normal)	37	31.1
	23 - 24.9 (Overweight)	22	17.6
	25 – 29.9 (Pre obese)	45	37.8
	30 or >30 (Obese)	16	13.5
Duration (Years)	≤ 3	37	31.6
	4 - 8	35	29.9
	≥ 9	45	38.5
Family history of Diabetes	Yes	84	70.0
	No	36	30.0

**Table II: Descriptive analysis of biochemical parameters**

Biochemical parameter	DM patients			Controls			p value
	Mean	SD	SEM	Mean	SD	SEM	
Blood Glucose (mg/dl)	163.03	50.89	4.64	95.24	10.18	1.95	< 0.001
Salivary Glucose 10x(mg/dl)	1.38	1.02	0.10	0.36	0.22	0.08	< 0.001
HbA1c (%)	8.78	2.31	0.30	5.40	0.55	0.11	< 0.001
Salivary Flow Rate (ml/minute)	0.30	0.15	0.02	0.25	0.15	0.04	0.282

SD: Standard Deviation SEM: Standard Error of Mean  $p < 0.05$ : significant

patients (Table III). Salivary glucose was significantly correlated with FBG and HbA1c levels in DM patients ( $r = 0.201$ ,  $p = 0.027$  and  $r = 0.288$ ,  $p = 0.031$  respectively). Correlation between FBG level and HbA1c level is statistically significant ( $p < 0.05$ ). However, no significant correlation was found between FBG and FSG in healthy controls (Table IV). Significant correlations were identified between salivary glucose with blood glucose in different onset periods of DM patients (Table V).

**Table III: Correlation between Biochemical parameters of diabetic mellitus patients (Pearson correlation)**

Biochemical parameter		Blood glucose	Salivary glucose	HbA1c	Salivary Flow Rate
Blood glucose	Correlation coefficient	-	0.201	0.432	0.110
	p value	-	0.027	0.001	0.315
Salivary glucose	Correlation coefficient	0.201	-	0.288	0.111
	p value	0.027	-	0.031	0.312
HbA1c	Correlation coefficient	0.432	0.288	-	0.113
	p value	0.001	0.031	-	0.426
Salivary Flow Rate	Correlation coefficient	0.110	0.111	0.113	-
	p value	0.315	0.312	0.426	-

$p < 0.05$ : significant Salivary Flow Rate (ml/minute)

Correlation graphs were plotted, and two formulas were derived for the prediction of blood glucose and HbA1c from salivary glucose (Figure 1, Figure 2). It shows positive relationships between FSG and FBS as well as FSG and HbA1c. By using the regression equation, we can predict the amount of FBG and HbA1c for a unit change in the salivary glucose.

### DISCUSSION

Diabetes mellitus is a metabolic disorder resulting from defects in insulin secretion by the pancreas and/or by the impotence of insulin action. Hyperglycemia leads to

**Table IV: Correlation between Biochemical parameters of healthy controls (Pearson correlation)**

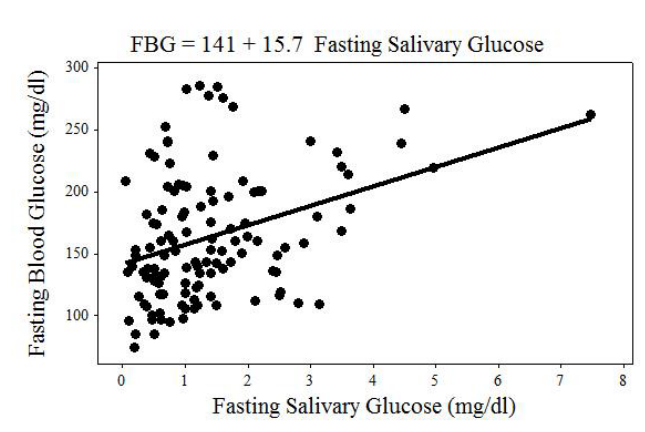
Biochemical parameter		Blood glucose	Salivary glucose	HbA1c	Salivary Flow Rate
Blood glucose	Correlation coefficient	-	0.151	0.495	0.313
	p value	-	0.451	0.019	0.156
Salivary glucose	Correlation coefficient	0.151	-	-0.186	0.014
	p value	0.451	-	0.408	0.408
HbA1c	Correlation coefficient	0.495	-0.186	-	0.168
	p value	0.019	0.951	-	0.456
Salivary Flow Rate	Correlation coefficient	0.313	0.014	0.168	-
	p value	0.156	0.408	0.456	-

p < 0.05: significant Salivary Flow Rate (ml/minute)

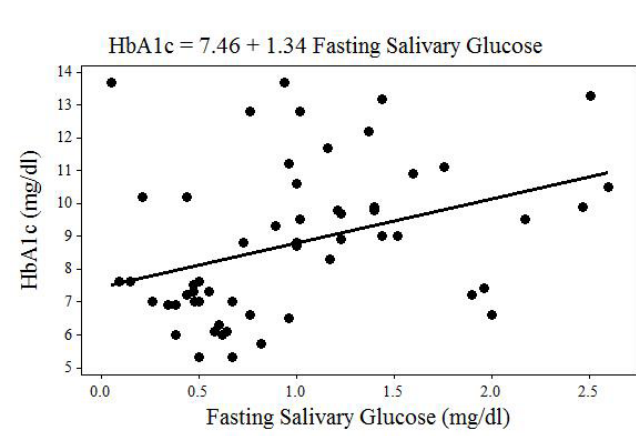
**Table V: Correlation of salivary glucose with blood glucose in different onset periods of diabetes mellitus patients**

Onset period (Years)	Variables	Correlation coefficient	p value
≤ 3	FSG vs FBG	0.511	0.001
	FSG vs HbA1c	0.502	0.028
	FSG vs SFR	0.034	0.880
4 - 8	FSG vs FBG	0.380	0.024
	FSG vs HbA1c	0.463	0.046
	FSG vs SFR	-0.178	0.384
≥ 9	FSG vs FBG	0.442	0.002
	FSG vs HbA1c	0.563	0.015
	FSG vs SFR	-0.034	0.840

FSG – Fasting salivary glucose; FBG – Fasting blood glucose; HbA1C – Hemoglobin A1C  
p < 0.05: significant Salivary Flow Rate (ml/minute)



**Figure 1: Linear regression model for Fasting Salivary Glucose and Blood Glucose levels in the DM patients**



**Figure 2: Linear regression model for Fasting Salivary Glucose levels and HbA1C levels in the DM patients**

several complications in the body by interfering almost all the regular body functions. Most of the people remain undiagnosed of DM as it does not show many symptoms. The blood sample collection procedure is invasive and cause many complications such as hematoma, dizziness, fainting, arterial puncture or laceration (19). A Non-invasive, complications less, patient friendly, accurate method is needed to avoid these complications (11). Several studies reported that there is a significant correlation exist between salivary and blood glucose and helpful to diagnosis and monitoring glucose level. Therefore, measurement of salivary glucose levels can be used as a non-invasive alternative method to overcome the problems arisen with invasive techniques such as taking blood to determine serum glucose level (20-23). The human saliva consists of water, electrolytes, enzymes, proteins, immunoglobulins, and biomarkers which can be useful for rapid tests (24,25). Therefore, it is suggested that the measurement of these components in saliva are important for future works to determine the diagnosis of diseases because saliva is an ultrafiltrate of blood and biochemical changes in the blood directly influence to saliva (20, 26, 27). Furthermore, methods of saliva collection are easy and non-invasive (24,26).

Saliva plays a large role for homeostasis in the oral cavity (20). The concentration of salivary glucose in diabetes patients was significantly higher than in healthy individuals. Similar results were reported in many areas worldwide (12,28,29). Salivary flow rate is not significantly correlated with blood and saliva glucose levels which were similar to several studies (30,31). However, a significant difference of mean values of salivary flow rate was reported in Type 2 diabetic patients compared to the healthy individuals (28). Healthy controls were used only to identify the range of salivary glucose and to establish the laboratory mean. However, these healthy controls were not age-matched with patients.

According to previous studies, salivary glucose values may be varying from person to person and highly

elevated in DM patients (12). However, age may be not contributed much as other parameters (eg. serum creatinine) and control subjects may not have much high values irrespectively the age. Therefore, in the present study it was observed huge variations of measured parameters among patients as well as controls.

In the present study, a significant correlation between FBG and FSG level was identified among DM patients. Our result is in good agreement with those reported by Gupta et al, 2015 (32) and Srikala et al, 2014 (33). Whole saliva contained relatively low levels of glucose comparing with the parotid secretion. However, in present study, we have found a higher significant correlation ( $P < 0.001$ ) between blood glucose and salivary glucose level of DM patients using whole saliva collected by the spitting method. Therefore, glucose level can be predicted using a formula with glucose level based on the linear regression analysis. However, several studies reported that patients with periodontal disease, food particles, bacteria and other potential contaminants in the oral cavity cause poor correlation between blood and saliva glucose levels in diabetic patients (34-36).

Present study observed that there was a high significant correlation between the HbA1c and salivary glucose. DM can be diagnosed by measuring blood HbA1c accurately without performing continuous blood glucose measurement routinely (37). The HbA1c level of a patient provides the information about glycemic control over the past 3 months. Similarly, several studies have reported that there were significant relationships between salivary glucose and HbA1c in DM patients (32,33).

Although studies have reported that glucose present in saliva under normal conditions, the mechanism of glucose secretion is still unknown (21). Hyperglycemia alters the normal structure of basement membrane in micro vessels causing more glucose infiltrate into the blood into the saliva in DM patients (38). Because glucose can easily diffuse through semi permeable membranes, more glucose moves from blood vessels to salivary glands in diabetes mellitus conditions. Further studies will focus the mechanism of salivary glucose secretion and the association between blood glucose level in DM patients.

## CONCLUSION

FSG was significantly correlated with FBS of diabetes and healthy individuals. FSG level may have potential to be used as a noninvasive method to determine the glycemic status of DM patients in the Sri Lankan clinical setup. However, further studies are needed to determine the mechanism of high glucose concentration in diabetes patients and to design noninvasive diagnostic tools for diabetes mellitus.

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