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Original Research

Comparison and Correlation of Glucose Levels in Serum and Saliva of Both Diabetic and Non-diabetic Patients

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Abstract:

Background: To detect and compare salivary glucose with plasma glucose level and postprandial blood sugar (PPBS) and fasting blood sugar (FBS) in diabetic and non-diabetic subjects.

Materials and Methods: A total of 100 patients were participated in this study. They were divided into two groups, each group consist of 50 patients. Un-stimulated saliva and blood were collected and investigated for glucose levels.

Results: FBS, PPBS, plasma glucose levels and salivary glucose levels were higher in diabetic patients than healthy controls. FBS, PPBS, plasma glucose level and salivary glucose levels were significantly correlated with each other in diabetic patients

Conclusion: Salivary glucose level can be used for monitoring tool to assess the glycemic status of diabetes mellitus patients as it is noninvasive and diagnostic method.

Key Words: Diabetes mellitus, hyperglycemia, saliva

Introduction

Saliva has many diagnostic uses and useful in both old and young patients. It is aloes useful in screening for various diseases and epidemiologic studies.[1,2] Due to this clinicians now tried various fluid other than blood and urine like saliva, sweat and tears for various diagnostic purposes.[1]

Saliva mainly consists of water, essential electrolytes, glucose, amylase, glycoproteins, antimicrobial enzymes.[3,4] Majority of molecules present in blood or urine can also be detected in salivary secretions, but in very less amount than those found in blood.[2] Saliva can be useful in estimation of glycemic control in patients with diabetes.

In diabetes mellitus relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues occurs. Altered salivary composition and function have been reported in diabetes mellitus. Due to this certain oral lesions are commonly seen in patients with diabetes mellitus such as a higher incidence of caries, periodontal disease and candidiasis.[5,6]

Basement membrane permeability of the parotid gland is reported to be higher in diabetes mellitus, and it results in increased percolation of components such as glucose, amylase and protein from blood occur due to increased permeability of basement membrane of parotid gland. Due to this glucose level in saliva increases. As glucose easily diffuses through the membrane of the blood vessels, it reaches to gingival sulcus through gingival fluid and blood serum and thus making its way to the saliva.[7]

Considering the limited amount of studies that evaluate the concentration of glucose in saliva and the controversy that exists in the relationship between salivary glucose and glycemia, in this study, we tried to evaluate correlation between concentration of salivary glucose, and plasma glucose in diabetic patients and in healthy patients.

Materials and Methods

With ethical approval from the Institutional Ethical Committee, the present study was carried out. In the study group, we have selected 50 diabetic patients randomly. We have also selected 50 healthy patients as controls. In diabetic patients both type 1 and type 2 diabetic patients were included.

Inclusion criteria

1. Any well-established cases of diabetes mellitus (either insulin dependent diabetes mellitus [IDDM]/non-insulin dependent diabetes mellitus [NIDDM]) diagnosed with features of polyuria, polydipsia, polyphagia and unexplained weight loss and elevated blood glucose levels or fasting blood sugar (FBS), as per the criteria established by the

Expert Committee on Diagnosis and Classification of Diabetes Mellitus in 1980.[8]
2. Patients should not take any medicines or insulin for diabetes mellitus.
3. For healthy control, patient should be non-diabetic and blood glucose levels should be within normal limits.
4. All the patients in this study should not have any other systemic disease.

Exclusion criteria

1. Patients with any other systemic diseases were excluded from the study.
2. Patients with severe diabetic complications were excluded from the study.
3. Patients on medications for diabetes were excluded.
4. Patients with habits of tobacco or alcohol were excluded from the study.
5. Patients with habits of smoking were excluded from the study.
6. Patients with current pregnancy were excluded from the study.

Before implementation of the study, informed written consents were obtained from all the participants at the beginning of this study. The data were obtained by self-administered brief medical history of patients through verbal questionnaire and the questionnaire was completed with detailed history of the patient.

Average plasma glucose, FBS, postprandial blood sugar (PPBS), salivary glucose were collected from both diabetic and healthy patients included in the study and statistical analysis was done using SPSS software.

FBS

FBS is measured by 8 h of whole night fasting. Based on Fox *et al.*[9] (Burket's Oral medicine 11th edition 2008) the FBS values are given below.

FBS level	Results
<110 mg/dl	Normal
110-126 mg/dl	Prediabetes (impaired fasting glucose)
≥126 mg/dl	Diabetes

PPBS

PPBS is measured after 2 h of a full meal. Based on Fox *et al.*[9] (Burket's Oral medicine 11th edition 2008) the PPBS values are given below.

PPBS level	Results
<140 mg/dl	Normal
140-200 mg/dl	Prediabetes (Impaired postprandial glucose)
≥200 mg/dl	Diabetes

Method of blood and saliva collection

The patients and control subjects were asked to come to the clinic in the morning around 8.00 a.m. to 10.00 a.m., on an empty stomach, after 8 h of fasting and 2 h after meal for blood collection.

Blood collection

Venipuncture, venipuncture, or phlebotomy is the process of obtaining intravenous access for the purpose of blood sampling of venous blood or for the intravenous therapy.

- Steps of phlebotomy procedure illustrated:
  - Patient identification
  - Filling out the requisition
  - Ready equipment
  - Apply tourniquet and palpate for vein
  - Sterilize the site
  - Insert needle
  - Drawing the specimen
  - Releasing the tourniquet
  - Applying pressure over the vein
  - Applying bandage
  - Disposing needle into sharps
  - Labeling the specimens

Saliva collection

Patients were asked to spit saliva (approximately 2 ml) into the sterile plastic saliva collection container. The collected saliva and blood samples were stored frozen until use in glucose assay. The saliva and blood samples were centrifuged. Estimation of glucose levels from the serum and supernatant saliva was done by using the glucose oxidase-peroxidase method (GOD-POD method).

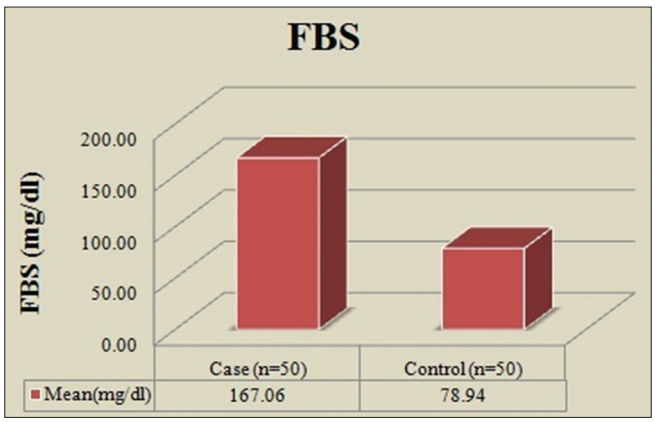
Serum and salivary glucose determination

Estimation of Serum and salivary glucose were done by the use of an enzymatic colorimetric test kit, by GOD-POD method (Glucose Kit, Erba Mannheim, Trinder's Method, Transasia Biomedical Ltd., HP, India). In GOP-POD method, the proportion is 1000 μL of reagent and 10 μL of saliva.

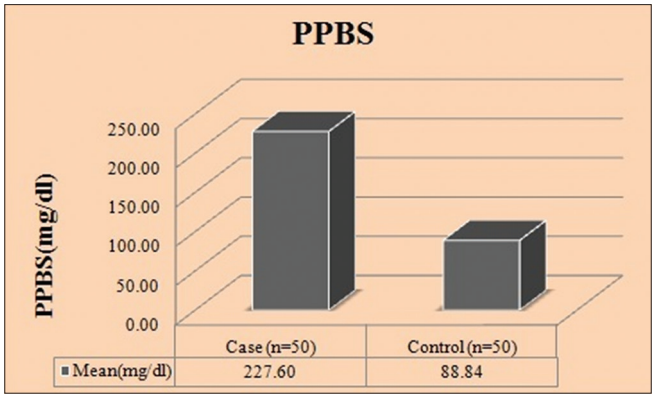
Results

In the study, total numbers of patients were 100 out of which 50 are diabetes patients, and 50 are healthy patients.

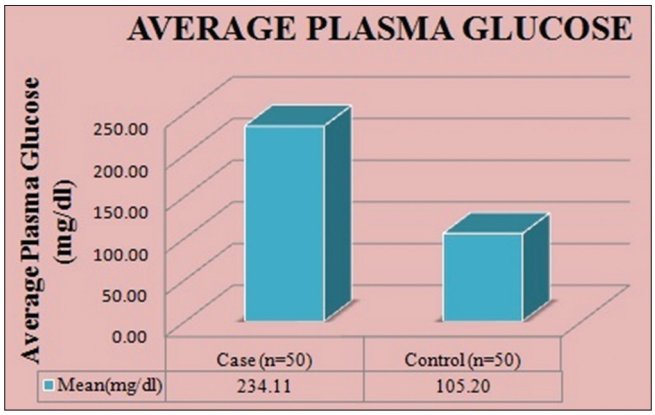
Mean FBS level in the diabetic patients group was higher (167.06 ± 57.24 mg/dl) than in healthy patients group (78.94 ± 16.70 mg/dl) (Graph 1). In a diabetic patients group, the mean PPBS was (227.60 ± 88.79 mg/dl) was higher than in healthy patients group (88.84 ± 22.86 mg/dl) (Graph 2). Mean average plasma glucose in diabetic patients was higher (234.11 ± 65.33 mg/dl) than healthy patients group (105.20 ± 24.77 mg/dl) (Graph 3). Similarly, the mean salivary glucose was higher in the diabetic patients group (13.96 ± 7.09 mg/dl) than in healthy patients (4.61 ± 2.58 mg/dl) (Graph 4). Thus, salivary glucose was



Graph 1: Correlation of mean fasting blood sugar level in diabetic patients and healthy controls.



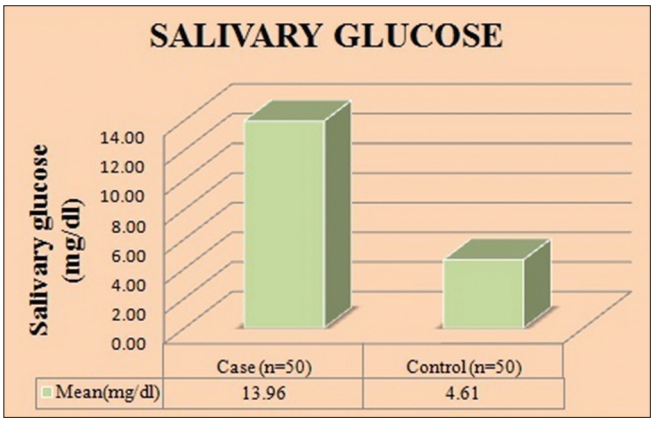
Graph 2: Correlation of mean postprandial blood sugar level in diabetic patients and healthy controls.



Graph 3: Correlation of mean average plasma glucose level in diabetic patients and healthy controls.

higher like higher FBS, PPBS, and average plasma glucose level in diabetic patients than in healthy patients.

Inter-group comparison of different variables shows a significant difference in mean of various variables like average plasma glucose, FBS, PPBS, and salivary glucose among diabetic patients and healthy patients. FBS, PPBS, Salivary glucose are significantly higher in diabetic patients than healthy patients (Table 1).



Graph 4: Correlation of mean salivary glucose level in diabetic patients and healthy controls.

Intragroup comparison of different variables like average plasma glucose, FBS, PPBS, salivary glucose were done in both diabetic and healthy patients (Tables 2 and 3).

Diabetic patients (Table 2)

Pearson's correlations coefficient and independent 2-tailed test showed that there were very high positive correlations of FBS with other variables - average plasma glucose, PPBS, salivary glucose. Similarly, PPBS also showed very high positive correlations of PPBS with other variables like average plasma glucose, FBS, salivary glucose. In case of salivary glucose, it showed a very high positive correlation with FBS and PPBS while the high positive correlation with average plasma glucose levels. Thus, very high positive intra-group correlations of different variables like average plasma glucose, FBS, PPBS, and salivary glucose in diabetic patients were recorded.

From our study, we have tried to predict the values of average plasma glucose, FBS, PPBS for a given salivary glucose by using following the regression equations in diabetic patients (Graphs 5-7).

1. Average plasma glucose = 7.115 × Salivary glucose + 134.8 (R^2 = 0.597)
2. HBA1c = 0.256 × Salivary glucose + 4.499 (R^2 = 0.843)
3. FBS = 7.432 × Salivary glucose + 63.33 (R^2 = 0.848)
4. PPBS = 11.68 × Salivary glucose + 64.49 (R^2 = 0.871)

Healthy patients (Table 3)

The Pearson's correlation and independent 2-tailed test performed for all variables like average plasma glucose, FBS, PPBS and salivary glucose were checked. Average plasma glucose has a high positive correlation with FBS, PPBS and salivary glucose. FBS showed a very high positive correlation with PPBS, high positive correlation with average plasma glucose, whereas positive correlation with salivary glucose. PPBS showed very high positive correlations with FBS, high positive correlation of with average plasma glucose and, whereas positive correlation with salivary glucose. Salivary

Table 1: Inter group comparison of different variables like average plasma glucose, FBS, PPBS, salivary glucose in diabetic patients and healthy patients.

Variables	Group	*N*	Mean	SD	SEM	Mean difference	*P* value	Result
Average plasma glucose (mg/dl)	Diabetic patients	50	234.11	65.33	9.24	128.91	<0.0001	S
	Healthy patients	50	105.20	24.77	3.50			
FBS (mg/dl)	Diabetic patients	50	167.06	57.24	8.09	88.12	<0.0001	S
	Healthy patients	50	78.94	16.70	2.36			
PPBS (mg/dl)	Diabetic patients	50	227.60	88.79	12.56	138.76	<0.0001	S
	Healthy patients	50	88.84	22.86	3.23			
Salivary glucose (mg/dl)	Diabetic patients	50	13.96	7.09	1.00	9.35	<0.0001	S
	Healthy patients	50	4.61	2.58	0.37			

n: Number of patients, SD: Standard deviation, SEM: Standard error of mean, s: Significant, FBS: Fasting blood sugar, PPBS: Postprandial blood sugar

Table 2: Intra group Pearson's correlations and independent 2-tailed tests of different variables like average plasma glucose, FBS, PPBS, salivary glucose in diabetic patients.

Variables	Average plasma glucose (mg/dl)	FBS (mg/dl)	PPBS (mg/dl)	Salivary glucose (mg/dl)
Average plasma glucose (mg/dl)				
Pearson correlation	1	0.887	0.855	0.773
Sig. (2-tailed)		0.000	0.000	0.000
FBS (mg/dl)				
Pearson correlation	0.887	1	0.978	0.921
Sig.(2-tailed)	0.000		0.000	0.000
PPBS (mg/dl)				
Pearson correlation	0.855	0.978	1	0.934
Sig. (2-tailed)	0.000	0.000		0.000
Salivary glucose (mg/dl)				
Pearson correlation	0.773	0.921	0.934	1
Sig. (2-tailed)	0.000	0.000	0.000	

FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, Sig.: Significant

Table 3: Intra group correlations of different variables like average plasma glucose, FBS, PPBS, salivary glucose in healthy patients.

Variables	Average plasma glucose (mg/dl)	FBS (mg/dl)	PPBS (mg/dl)	Salivary glucose (mg/dl)
Average plasma glucose (mg/dl)				
Pearson correlation	1	0.745	0.721	0.706
Sig. (2-tailed)		0.000	0.000	0.000
FBS (mg/dl)				
Pearson correlation	0.745	1	0.953	0.481
Sig. (2-tailed)	0.000		0.000	0.000
PPBS (mg/dl)				
Pearson correlation	0.721	0.953	1	0.535
Sig. (2-tailed)	0.000	0.000		0.000
Salivary glucose (mg/dl)				
Pearson correlation	0.706	0.481	0.535	1
Sig. (2-tailed)	0.000	0.000	0.000	

FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, Sig.: Significant

glucose showed a high positive correlation with average plasma glucose, whereas positive correlation with FBS, PPBS.

Thus, there are high positive intra-group correlations of different variables like average plasma glucose, FBS, PPBS and salivary glucose were noted in healthy patients. Thus in diabetic patients there are a very high positive correlation of different variables, whereas in the healthy patient only high positive correlations were noted.

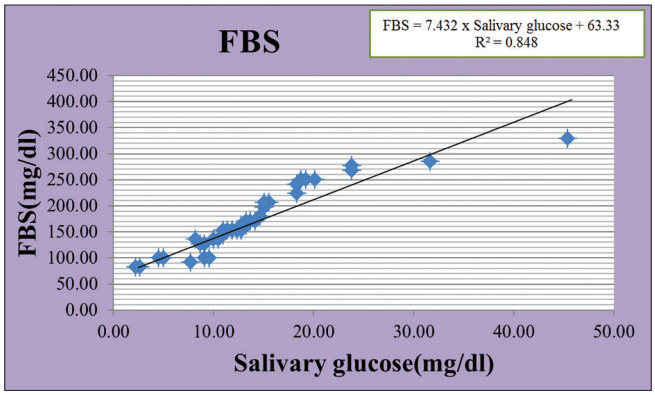
Discussion

In diabetes mellitus relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues occurs.[10]

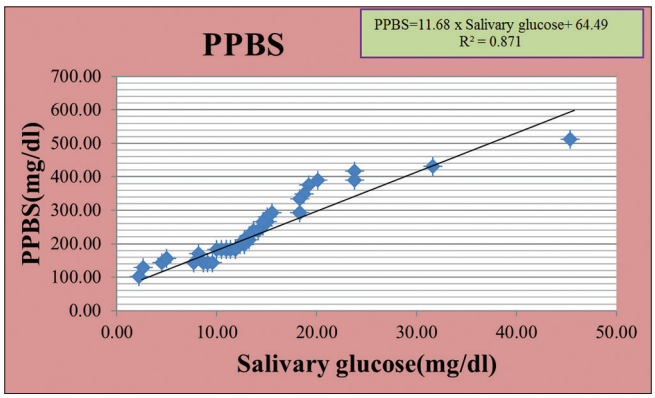
The prevalence among adults aged 20-70 years is expected to rise from 285 million (2010) to 438 million by the year 2030. The present trend indicates that more than 60% of the world's diabetic population will be in Asia.[11]

The most recognized types of diabetes are type 1 (IDDM) and type 2 (NIDDM). The other types of diabetes are gestational diabetes, secondary diabetes, and maturity-onset diabetes of the young.[12] Altered salivary composition and function commonly found in patients with diabetes mellitus.[5]

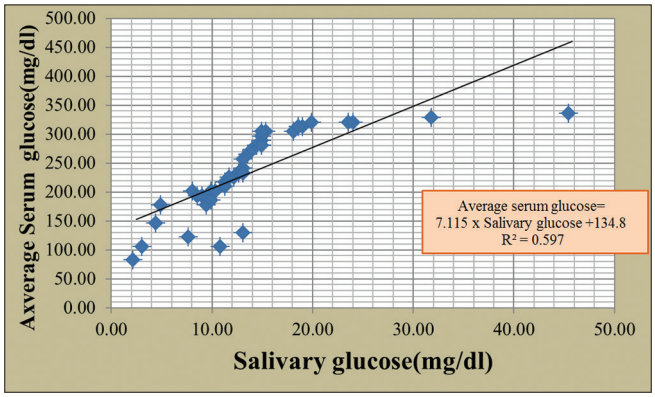
In our study, we found that salivary glucose values were higher among diabetics (13.96 ± 7.09) than in the controls (4.61 ± 2.58); the difference was statistically highly significant



Graph 5: Correlation between salivary glucose and fasting blood sugar in diabetic patients.



Graph 6: Correlation between salivary glucose and postprandial blood sugar in diabetic patients.



Graph 7: Correlation between salivary glucose and average serum glucose in diabetic patients.

($P < 0.0001$). Whereas, Vasconcelos *et al.* (2010) reported nonsignificant difference in the mean salivary glucose level in diabetic patients (14.03 ± 16.76 mg/dl) and in the control group (6.35 ± 6.02 mg/dl) ($P = 0.036$).[7] Karjalainen *et al.* (1996) mentioned that in the newly diagnosed IDDM cases, mean salivary glucose level decreased from 54.1 ± 31.7 mg/l to 35.2 ± 29.5 mg/l ($P = 0.096$) after beginning insulin treatment.[13]

Darwazeh *et al.* (1991), Karjalainen *et al.* (1996), Panchbhai *et al.* (2010), and Amer *et al.*[15] (2001) mentioned that salivary glucose concentration was significantly higher in diabetic patients than in the healthy controls.[5,13-15] Soares *et al.* (2009) did a study only on the determination of salivary glucose in healthy adults, and found glucose is present in the saliva of healthy individuals and the concentration of salivary glucose did not present any statistically significant correlation with capillary glycemia.[16] Takai *et al.* (1983) have reported that glucose is also present in the saliva of normal individuals.[17] They proposed paracellular and intercellular pathways.[18] In our study, significant correlations was established between salivary glucose with average plasma glucose, FBS and PPBS in diabetic patients.

Similar to our study, Abikshyeet *et al.* (2012) mentioned that was a positive and statistically significant correlation between salivary and plasma glucose in diabetic patients.[19] Hence, salivary glucose can be useful in prediction of plasma glucose concentration in diabetic patients.

Reuterving *et al.* (1987) mentioned that in eleven diabetics (eight with type 1 diabetes) there was a positive correlation present between glucose levels in saliva and blood.[20] During the period of better metabolic control salivary glucose concentration reported lower. Thus, the degree of diabetic metabolic control significantly affects the salivary glucose concentration. Amer *et al.* (2001) mentioned that in type 2 diabetic patients, significant correlation was found between serum and salivary glucose concentration.[14] They mentioned that the patient had average elevated blood glucose concentration level over an extended period of time, and glucose was only found in the saliva of diabetic individuals. Non-diabetic individuals did not show the presence of glucose in saliva. Belazi *et al.* (1998) mentioned that significantly greater concentrations of glucose were seen in saliva and serum of children with IDDM.[21] Thus, estimated salivary glucose levels, play a helpful role in the diagnosis of IDDM in some children in early stages.

However, in contrast to our study, Forbat *et al.* (1981) and Carda *et al.* (2006) showed no significant correlation between salivary and plasma glucose.[22,23] In these studies old methods were used for glucose determination which was not appropriate. Even Englander *et al.* (1963) expressed doubt regarding replacement of serum with parotid secretion in the diagnosis of diabetes mellitus, because of its lower levels of glucose concentration.[24] However, Mitsumori *et al.* (1998) manufactured a saliva analyzing system using a glucose sensor and performed *in vivo* evaluations, concluding that their salivary glucose level measurement system could be used as an indicator of blood glucose level.[25] The variability in the results of different workers may be a reflection of different choice of study design, as well as the diversity of the methods and criteria for selecting the samples.[16]

According to Feller and Shannon (1975), correlation with salivary glucose was found both in fasting and postprandial.[26]

Abikshyeet *et al.* (2012) in his study showed a salivary glucose levels increase as plasma glucose level increased.[19] López *et al* (2003) showed that the salivary glands act as filters of blood glucose which may be altered by hormonal or neural regulation.[27] According to Qureshi *et al.* (2007) showed that there is increased leakage of glucose from the ductal cells of the salivary gland, so salivary glucose level is increased in diabetic patients.[28] This is due to microvascular changes in blood vessels and change in the basement membrane in diabetic patients.[29] Hyperglycemia leads to increased formation of advanced glycosylation end (AGEs) products. These AGEs products crosslink proteins such as collagen and extracellular matrix proteins, leading to basement membrane alteration and endothelial dysfunction, which makes them more permeable. This permeability is also increased by other products, such as sorbitol, diacylglycerol, and fructose-6-phosphate, which are formed because of chronic hyperglycemia, which explains the increased passage of glucose from the blood into the saliva in diabetes mellitus.[29] This is supported by Belazi *et al.* who proposed that the increased permeability of basement membrane in diabetic patients may lead to enhanced leakage of smaller molecules like glucose into whole saliva via gingival crevices.[21] Due to this increased glucose levels were reported in salivary secretion of patients with diabetes mellitus. The presence and increase of glucose levels in saliva is multifactorial and no single mechanism can be responsible in diabetic patients and non-diabetic patients.

Conclusion

We found increased salivary glucose levels in patients having diabetes mellitus. Salivary glucose level can be used for monitoring tool to assess the glycemic status of diabetes mellitus patients as it is noninvasive and diagnostic compare to other fluids. Further studies with larger populations in different geographic areas are needed to establish salivary glucose estimation as an excellent diagnostic as well as a monitoring tool for diabetes mellitus.

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