

# Correlation of salivary glucose, blood glucose and oral candidal carriage in the saliva of type 2 diabetics: A case-control study

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## Abstract

**Objectives:** To study the correlation between blood glucose levels and salivary glucose levels in type 2 diabetic patients, to study the relationship between salivary glucose levels and oral candidal carriage in type 2 diabetic patients and to determine whether salivary glucose levels could be used as a noninvasive tool for the measurement of glycemic control in type 2 diabetics. **Study Design:** The study population consisted of three groups: Group 1 consisted of 30 controlled diabetics and Group 2 consisted of 30 uncontrolled diabetics based on their random nonfasting plasma glucose levels. Group 3 consisted of 30 healthy controls. Two milliliters of peripheral blood was collected for the estimation of random nonfasting plasma glucose levels and glycosylated hemoglobin (HbA1c). Unstimulated saliva was collected for the estimation of salivary glucose. Saliva was collected by the oral rinse technique for the estimation of candidal counts. **Results:** The salivary glucose levels were significantly higher in controlled and uncontrolled diabetics when compared with controls. The salivary candidal carriage was also significantly higher in uncontrolled diabetics when compared with controlled diabetics and nondiabetic controls. 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 control in diabetic patients. **Conclusion:** The present study found that estimation of salivary glucose levels can be used as a noninvasive, painless technique for the measurement of diabetic status of a patient in a dental set up. Increased salivary glucose levels leads to increased oral candidal carriage; therefore, oral diagnosticians are advised to screen the diabetic patients for any oral fungal infections and further management.

**Keywords:** HbA1c, oral candidal carriage, salivary glucose levels, type 2 diabetics, type 2 diabetes mellitus

## Introduction

Diabetes mellitus is a complex multisystemic metabolic disorder characterized by a relative or absolute deficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues.<sup>[1]</sup> Diabetes can be divided into two types based on the age group. In type 1 diabetes, the  $\beta$ -cells of pancreas are destroyed by the autoimmune mechanism. In Type 2 diabetes, a resistance to

insulin is developed.<sup>[1]</sup> Criteria for the diagnosis of diabetes mellitus include one of the following:<sup>[2]</sup>

1. A1C  $\geq$  6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay
2. FPG  $\geq$  126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h
3. 2-h plasma glucose  $\geq$  200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose  $\geq$  200 mg/dL (11.1 mmol/L).

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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. Studies suggest that diabetics are more susceptible to fungal infections in areas of moisture and trauma. Candidiasis is one of the most common infections encountered in the oral cavity of diabetics.<sup>[1-4]</sup> Saliva is an organic fluid that is easy to collect by noninvasive means and can easily be preserved. Several studies have shown that salivary glucose levels can be used as an important noninvasive indicator of the blood glucose levels.<sup>[1]</sup> Hence, the present study was being undertaken to detect the salivary glucose levels in diabetic and nondiabetic patients, to study the relationship between salivary glucose

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levels and salivary candidal carriage and also to determine whether salivary glucose levels could be used as a noninvasive tool to monitor glycemic control in diabetics.

## Materials and Methods

### Study population

All known type 2 diabetic patients visiting the outpatient department of the Vydehi Institute of Dental Sciences and the Diabetic Clinic-Vydehi Institute of Medical Sciences were included in the study. The ethical clearance for the study was obtained from the ethical committee of the Vydehi Institute of Dental Sciences. The patients with any other systemic diseases, recent history and/or currently on medications that affect the oral microflora and salivary flow were excluded. Patients with complaints of xerostomia or with a habit of using smoking and smokeless forms of tobacco and alcohol and patients using dentures were also excluded from the study.

The study population consisted of three groups. Group 1 consisted of 30 controlled diabetics whose random nonfasting plasma glucose levels were in the range of 120-200 mg/dL. Group 2 consisted of 30 uncontrolled diabetics with random nonfasting plasma glucose levels greater than 200 mg/dL. Group 3 consisted of 30 healthy controls with random nonfasting plasma glucose levels in the range of 80-120 mg/dL.

### Blood glucose and glycosylated hemoglobin estimation

The case history was recorded and a detailed intraoral examination was performed, following which 2 mL of peripheral blood was collected from every patient for the estimation of random nonfasting plasma glucose levels and glycosylated hemoglobin. The random nonfasting plasma glucose levels were measured using the glucose oxidase method. Glycosylated hemoglobin levels were measured using the ion exchange resin method.

### Saliva collection for glucose estimation

Saliva was collected for glucose estimation. All salivary samples were collected 2 h 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 speak, swallow or do any head movements 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.<sup>[5]</sup>

Salivary glucose levels were measured using the glucose oxidase method in a semiautomated analyzer. The saliva sample (100  $\mu$ L) was mixed with the reagent (glucose oxidase) in a 1:3 ratio 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 0.2 mg/dL.<sup>[5]</sup>

### Saliva sampling and assessment of salivary candidal count

Saliva collection for the measurement of candidal count was performed using the oral rinse technique. All subjects rinsed their mouth for 60 s with 10 mL sterile phosphate-buffered saline. Each subject returned the rinse to a sterile container. The salivary rinse was concentrated by centrifuging at 1700 rpm for 10 min. The supernatant was discarded and 0.001  $\mu$ L inoculating loop was used to spread the sample on to Sabouraud dextrose agar plates, supplemented with chloramphenicol (10 mg/dL). Colony-forming units (CFUs) were counted manually and the number was multiplied by 1000 and expressed as CFU/mL.<sup>[5]</sup>

### Statistics

Analysis of variance (ANOVA) has been used to determine the significance of the study parameters between three or more groups of patients, while the Chi-square/Fisher Exact test was used to find the significance of study parameters on a categorical scale between two or more groups. The Pearson correlation ( $r$ ) and t-test of a correlation coefficient were performed. Diagnostic statistics were applied to evaluate the sensitivity and specificity of the test. The values based on the diagnostic statistics are depicted as the area under the curve in the receiver operating characteristic. The Statistical software, namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver. 2.11.1, were used for the analysis of the data and Microsoft Word and Excel were used to generate graphs, tables, etc.

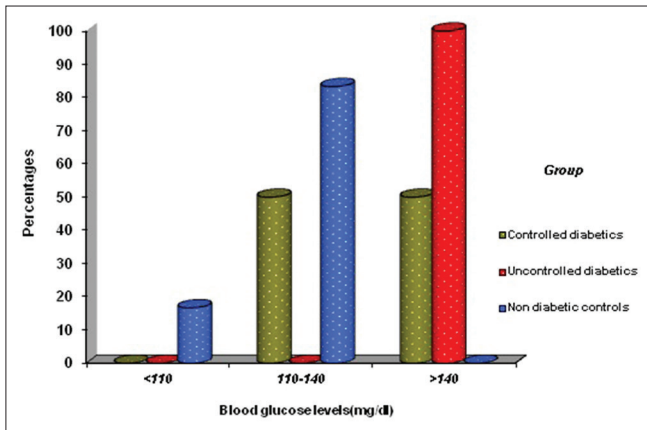
## Results

In our study, when the distribution of random nonfasting plasma glucose levels in the three groups were studied, the blood glucose levels varied between 120-200 mg/dL, >200 mg/dL and 80-120 mg/dL [Table 1 and Figure 1]. The glycosylated hemoglobin levels in the three groups varied between 6.2-7.1%, 7.4-12.8% and 4.5-5.3% [Table 2 and Figure 2]. The salivary glucose levels among the three groups varied between 7.4-17.8 mg/dL, 10.4-15.4 mg/dL and 2.2-6.8 mg/dL [Table 3 and Figure 3]. The salivary candidal counts in the three groups varied between 600-8400, 1500-23,200 and 400-4000 [Table 4 and Figure 4].

In controlled diabetics, the salivary glucose levels showed a very large correlation coefficient ( $r = 0.841$ ) and statistically strong significant association ( $P < 0.001$ ) with blood glucose levels. A large correlation coefficient ( $r$ -value = 0.629) and statistically strong significant association ( $P < 0.001$ ) was seen between salivary glucose levels and glycosylated hemoglobin. The correlation coefficient between salivary glucose levels and candidal counts showed a large correlation ( $r = 0.539$ ) in controlled diabetics. A statistically significantly strong association was seen between salivary glucose levels and candidal counts ( $P < 0.001$ ) in the same [Table 5 and Figure 5].

**Table 1: Distribution of Salivary glucose levels (mg/dl) in three groups of patients studied**

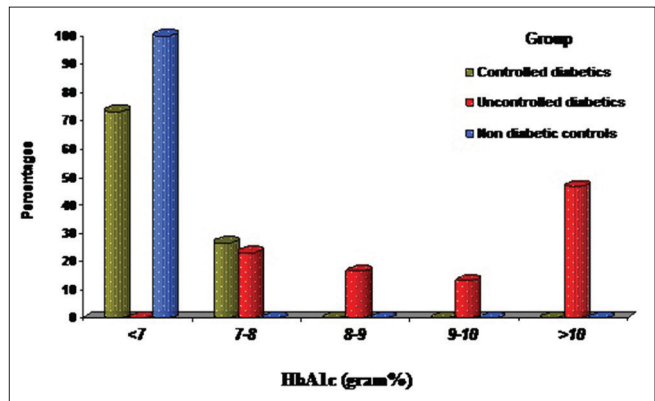
Salivary glucose levels (mg/dl)	Group (%)		
	Controlled diabetics (RBS 120-200 mg/dl)	Uncontrolled diabetics (RBS>200 mg/dl)	Non diabetic controls (RBS 80-120 m/dl)
<4.1	0 (0.0)	0 (0.0)	11 (36.7)
4.1-13.3	18 (60.0)	13 (43.3)	19 (63.3)
>13.3	12 (40.0)	17 (56.7)	0 (0.0)
Total	30 (100)	30 (100)	30 (100)
Inference	Salivary glucose levels are significantly more in uncontrolled diabetics followed by controlled diabetics with $P < 0.001^{**}$		



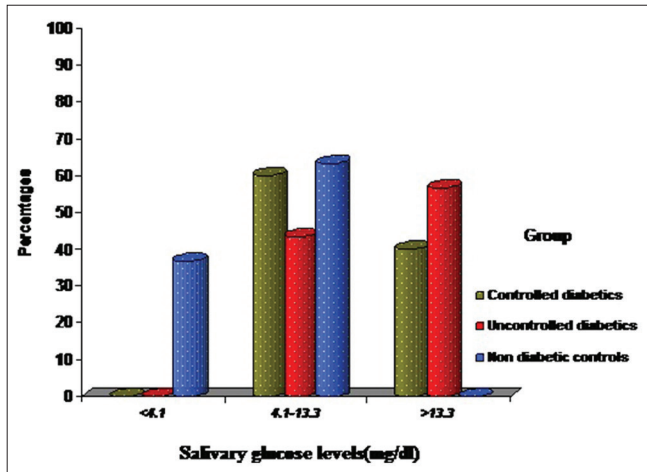
**Figure 1: Blood glucose levels**

**Table 2: Distribution of HbA1c (gram%) in three groups of patients studied**

HbA1c (gram%)	Group (%)		
	Controlled diabetics (RBS 120-200 mg/dl)	Uncontrolled diabetics (RBS>200 mg/dl)	Non diabetic controls (RBS 80-120 m/dl)
<7	22 (73.3)	0 (0.0)	30 (100)
7-8	8 (26.7)	7 (23.3)	0 (0.0)
8-9	0 (0.0)	5 (16.7)	0 (0.0)
9-10	0 (0.0)	4 (13.3)	0 (0.0)
>10	0 (0.0)	14 (46.7)	0 (0.0)
Total	30 (100)	30 (100)	30 (100)
Inference	HbA1c levels are significantly more in uncontrolled diabetics with $P < 0.001^{**}$		

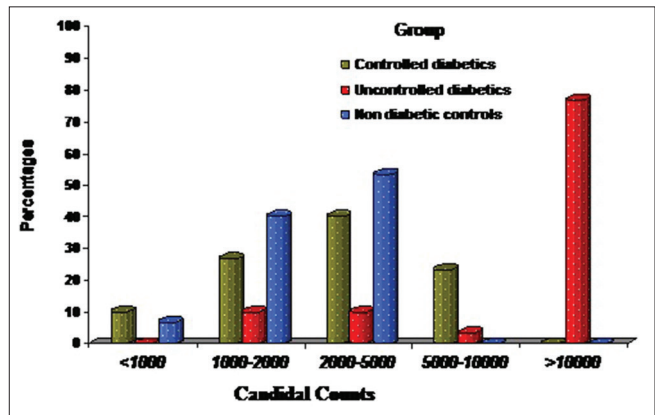


**Figure 2: Glycosylated hemoglobin levels**



**Figure 3: Salivary glucose levels**

In uncontrolled diabetics, the salivary glucose levels show a nearly perfect correlation coefficient ( $r = 0.929$ ) and a statistically strong significant association ( $P < 0.001$ ) with blood glucose levels. A nearly perfect correlation coefficient ( $r = 0.906$ ) and a statistically strong significant association ( $P < 0.001$ ) was seen between salivary glucose levels and glycosylated hemoglobin. The correlation coefficient between salivary glucose levels and candidal counts



**Figure 4: Salivary candidal counts**

showed a very large correlation ( $r = 0.743$ ). A statistically strong association was seen between salivary glucose levels and candidal counts ( $P < 0.001$ ) [Table 5 and Figure 5].

In controls, the salivary glucose levels showed a moderate correlation coefficient ( $r = 0.370$ ) and suggestive significance ( $P = 0.044$ ) with blood glucose levels. This shows that the blood glucose levels might increase with an increase in the salivary glucose levels. A small correlation coefficient ( $r = 0.217$ )

and no statistically significant association ( $P = 0.249$ ) was seen between salivary glucose levels and glycosylated hemoglobin. The correlation coefficient between salivary glucose levels and candidal counts showed a moderate correlation ( $r = 0.333$ ). A statistically suggestive association was seen between salivary glucose levels and candidal counts ( $P = 0.072$ ) [Table 5 and Figure 5].

## Discussion

Results show an increase in the salivary glucose levels in controlled and uncontrolled diabetics as compared with control patients. These findings are similar to the findings of Andersson *et al.*, who also observed that salivary glucose levels were higher in diabetic subjects than in nondiabetic subjects after oral glucose loading.<sup>[6]</sup> Salivary glucose concentration did not significantly correlate with serum glucose, as seen in the study conducted by Maria soares.<sup>[7]</sup> In controlled diabetics, salivary glucose levels show a very

large correlation coefficient ( $r = 0.841$ ) and statistically strong significant association ( $P < 0.001$ ) with blood glucose levels. The study of Kortuem and Shannon *et al.* reported a rise in the salivary glucose following the rise in blood glucose after a glucose load suggestive of similar results as obtained in our study.<sup>[8,9]</sup> Forbet *et al.* concluded from their study that salivary glucose levels did not reflect blood glucose levels.<sup>[10]</sup> The salivary samples of the nondiabetic control subjects did not show the presence of glucose even in the slightest concentrations, while the samples obtained from the diabetics showed significant concentrations of glucose in the saliva in the study performed by Shehla *et al.*, which is the same as obtained in our study [Figure 6].<sup>[11]</sup> Belazi *et al.* proposed that the increased permeability of basement membrane in insulin-dependent diabetes mellitus may lead to enhanced leakage of serum-derived components in whole saliva via gingival crevices. The small glucose molecule can easily diffuse via the semipermeable basement membrane, resulting in increased salivary glucose levels.<sup>[12]</sup> In a study conducted by Kannan *et al.*, it was observed that salivary glucose levels were more in controlled and uncontrolled diabetics when compared with nondiabetic controls, which is similar to our study.<sup>[5]</sup> This finding suggests that salivary glucose levels likely follow a threshold mechanism. This increase in salivary glucose levels with increase in blood glucose levels has been suggested to be attributed to “leakage” across the basement membrane of the glands, particularly the parotid gland, when blood glucose levels increase beyond a threshold value.<sup>[5]</sup> Therefore, we can infer that in nondiabetics, because of the absence of the disease process, there is no change in the basement membrane permeability leading to less amounts of glucose in saliva as seen in our study [Figure 7].

A large correlation coefficient ( $r = 0.629$ ) and statistically strong significant association ( $P < 0.001$ ) was seen between

**Table 3: Distribution of Candidal Counts in three groups of patients studied**

Candidal counts	Group (%)		
	Controlled diabetics (RBS 120-200 mg/dl)	Uncontrolled diabetics (RBS>200 mg/dl)	Non diabetic controls (RBS 80-120 m/dl)
<1000	3 (10)	0 (0.0)	2 (6.7)
1000-2000	8 (26.7)	3 (10.0)	12 (40.0)
2000-5000	12 (40.0)	3 (10.0)	16 (53.3)
5000-10000	7 (23.3)	1 (3.3)	0 (0.0)
>10000	0 (0.0)	23 (76.7)	0 (0.0)
Total	30 (100)	30 (100)	30 (100)
Inference	More candidal counts are significantly more associated with uncontrolled diabetics with $P < 0.001^{**}$		

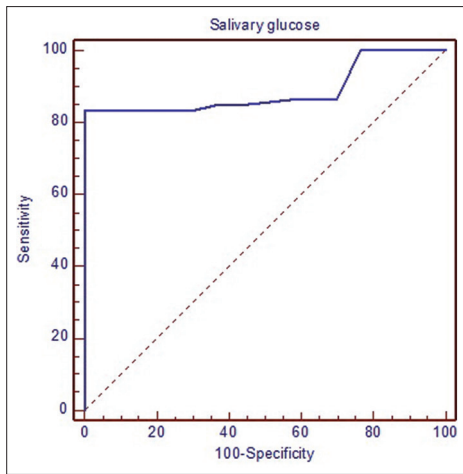
**Table 4: P-values**

Pair	Controlled diabetics		Uncontrolled diabetics		Non diabetic controls	
	r value	P value	r value	P value	r value	P value
Salivary glucose levels (mg/dl) vs Blood glucose levels (mg/dl)	0.841	<0.001**	0.929	<0.001**	0.370	0.044*
Salivary glucose levels (mg/dl) vs HbA1c (gram %)	0.629	<0.001**	0.906	<0.001**	0.217	0.249
Salivary glucose levels (mg/dl) vs Log Candidal Counts	0.539	<0.001**	0.743	<0.001**	0.333	0.072+

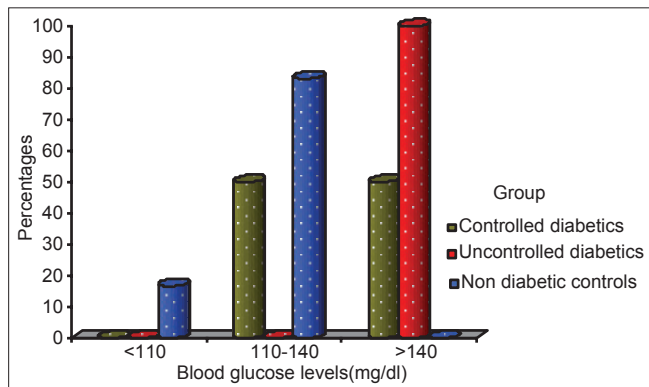
**Table 5: Comparison of study variables in three groups of patients studied**

Variables	Controlled diabetics (Group 1)	Un-controlled diabetics (Group 2)	Non diabetic controls (Group 3)	Significance		
				Controlled diabetics- Un-controlled diabetics	Controlled diabetics- Non diabetic controls	Un-controlled diabetics- Non diabetic controls
Blood glucose levels (mg/dl)	151.13±23.66	355.83±77.38	112.60±4.11	<0.001**	0.006**	<0.001**
Salivary glucose levels (mg/dl)	11.87±3.01	13.34±1.61	4.58±1.32	0.023*	<0.001**	<0.001**
HbA1c (gram %)	6.68±0.29	9.99±1.96	4.81±0.25	<0.001**	<0.001**	<0.001**
Log Candidal counts	7.90±0.77	9.34±0.83	7.59±0.55	<0.001**	0.236	<0.001**

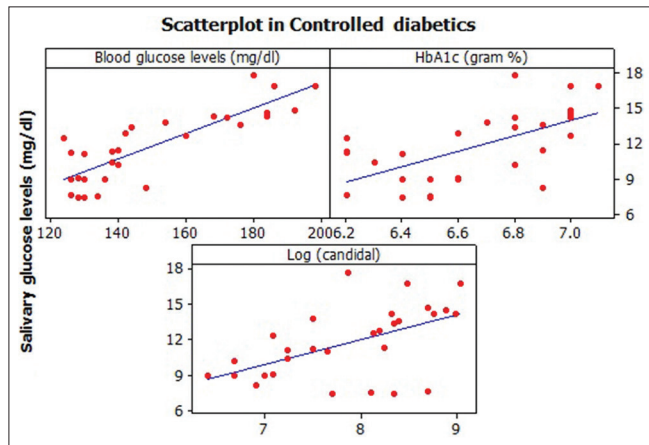




**Figure 5:** Receiver operator characteristic curve

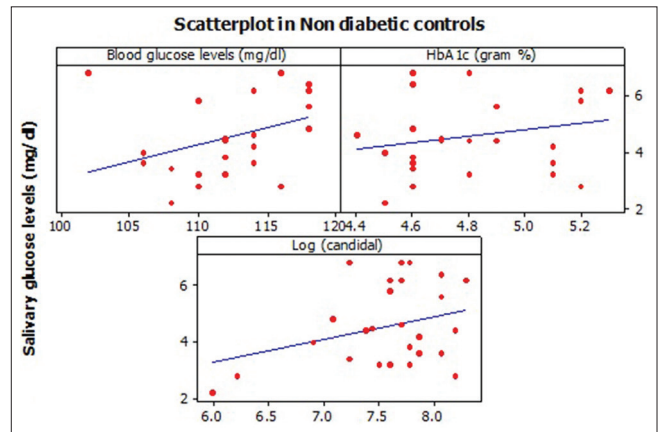


**Figure 7:** Blood glucose levels in the three groups

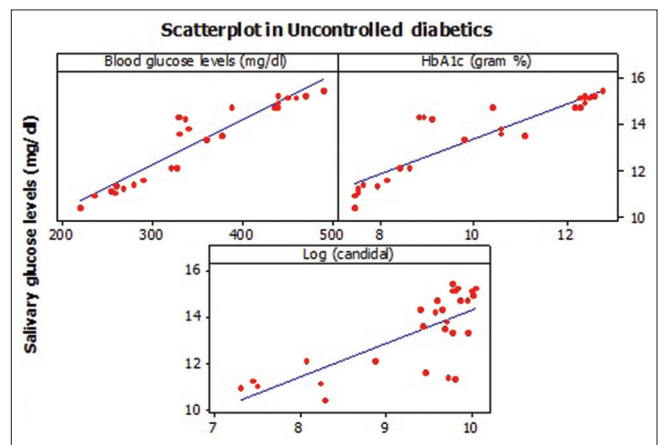


**Figure 9:** Scatter plot for controlled diabetics

salivary glucose levels and glycosylated hemoglobin in controlled diabetics. A study conducted by Panda *et al.* showed a high correlation between salivary glucose levels and glycosylated hemoglobin in controlled diabetics, which is similar to our study.<sup>[13]</sup> In contrast to our study, Lopez *et al.* did not find any correlation between salivary glucose level and HbA1c percentage.<sup>[14]</sup> The HbA1c levels did not show a



**Figure 6:** Scatter plot in nondiabetic controls



**Figure 8:** Scatter plot in uncontrolled diabetics

correlation with salivary glucose levels in groups 2 and 3, probably reflecting the fact that it represents an average value for 3 months.<sup>[5]</sup>

The correlation coefficient between salivary glucose levels and candidal counts showed a large correlation ( $r = 0.539$ ) in controlled diabetics. A statistically significant strong association was seen between Log salivary glucose levels and candidal counts ( $P < 0.001$ ). These findings suggest that as blood glucose levels increase the salivary glucose levels, glycosylated hemoglobin levels and the candidal counts also increase in controlled diabetics. Increased candidal density was seen more commonly in diabetics than in nondiabetics in a study conducted by Knight, which was similar to our study.<sup>[15]</sup> The oral carriage of *Candida* in diabetic patients was independent of glycemic control in a study conducted by Fischer *et al.*, which refutes our study.<sup>[16]</sup> Salivary glucose levels show a nearly perfect correlation coefficient ( $r = 0.929$ ) and statistically strong significant association ( $P < 0.001$ ) with blood glucose levels in uncontrolled diabetics. A nearly perfect correlation coefficient ( $r = 0.906$ ) and statistically strong significant association ( $P < 0.001$ ) was seen between salivary glucose levels and glycosylated

**Table 6: Diagnostic potential of Salivary glucose for predicting the diabetics**

Salivary glucose	Sensitivity	Specificity	AUC	P value
>6.8	83.33	100.00	0.888	<0.001**

hemoglobin in uncontrolled diabetics [Figure 8]. HbA1c reflects the average blood glucose concentration over an extended period of time, and remains unaffected by short-term fluctuations in blood sugar levels.<sup>[5]</sup> HbA1c levels in these patients correlated significantly with their serum glucose concentrations, which is similar to our study.<sup>[5]</sup> The correlation coefficient between salivary glucose levels and candidal counts showed a very large correlation ( $r = 0.743$ ) in uncontrolled diabetics. A statistically significant strong association was also seen between salivary glucose levels and candidal counts ( $P < 0.001$ ) in uncontrolled diabetics. A study performed by Hill *et al.* showed that very poorly controlled diabetics or uncontrolled diabetics are significantly more susceptible to have oral candidiasis, which is similar to our study.<sup>[17]</sup> Salivary glucose forms chemically reversible glycosylation products with proteins in tissues during hyperglycemic episodes, and this leads to the accumulation of glycosylation products on the buccal epithelial cells, which in turn may increase the number of available receptors for *Candida*.<sup>[17]</sup> This finding is suggestive of the fact that uncontrolled or poorly controlled diabetes increases the susceptibility to oral opportunistic infections, such as oral candidiasis.<sup>[18]</sup>

The diagnostic performance of salivary glucose levels to predict the blood glucose levels was performed by evaluating the sensitivity and specificity, according to which the probability of detecting diabetes by measuring salivary glucose levels (sensitivity) was 83.33%. The specificity that is the probability of detecting the absence of diabetes was 100%. The area under curve is 0.888 and the  $P < 0.001$ , suggesting a statistically strong significance. Because the area under the curve is 0.888, it infers from our study that the estimation of salivary glucose levels is a good test for measuring glycemic control in diabetes [Table 6 and Figure 9].

## Conclusion

From this study, we can conclude that the detection of diabetes can be performed by measuring the salivary glucose levels. This study can be taken as a stepping stone to establishing accepted reference values for salivary glucose, whereby, in the future, saliva may serve as a non-invasive specimen for the detection of diabetes. Oral diagnosticians are advised to screen the diabetic patients for any oral

fungal infections at the earliest and further institute the management for the same.

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