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Original Article

Accuracy of salivary glucose assessment in diagnosis of diabetes and prediabestes

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ABSTRACT

Aims: to assess salivary glucose correlation with blood glucose and its accuracy in diagnosis of diabetes and prediabetes.
Materials and methods: A comparative study including 204 adults in 3 groups (104 type 2 diabetics, 50 prediabetics, 50 non-diabetic controls) aging 18–65 years. The participants were interviewed about their socio-demographic, comorbidities, & drug treatment using a predesigned questionnaire. Salivary & blood samples were collected and analyzed.
Results: Mean salivary glucose was observed to be 23.40 ± 12.755 mg/dl in control group, 42.68 ± 20.830 mg/dl in prediabetic group and 59.32 ± 19.147 mg/dl in diabetic group with significant difference between the 3 groups (P value < 0.001). Salivary glucose was significantly correlated to FBS with strong positive association (r = 0.67, P value < 0.001 in control group, r = 0.56, P value < 0.001in diabetic group and r = 0.36, P value 0.01 in pre-diabetic group). Salivary glucose could differentiate non-diabetics from diabetics (AUC: 0.928, P value < 0.001) with sensitivity (94.2%) and specificity (62%) & differentiate non-diabetics from prediabetics (AUC: 0.928, P value < 0.001) with sensitivity (94.2%) and specificity (62%).
Conclusions: Salivary glucose estimation can serve as valid and non-invasive test for screening and diagnosis of diabetes & prediabetes.

1. Introduction

Diabetes is documented as the chronic disease with highest prevalence in the century by the World Health Organization and the International Diabetes Federation. In Egypt, there were over 7.8 million cases of diabetes in 2015. Over 40.6% of these are undiagnosed [1].

The prevalence of prediabetes is growing all over the world and it is anticipated that >470 million people will have prediabetes in 2030. Prediabetes is linked to kidney, retina & peripheral nerve affections and cerebrovascular attacks [2].

The most commonly used laboratory diagnostic techniques require the analysis of blood, but other biological fluids are also being utilized for the diagnosis of many diseases and of these, saliva provides peculiar advantages [3].

The salivary glands are exocrine glands that secret the salivary fluid which is formed of 99% water and other components like electrolytes, proteins, nitrogenous products, and glucose in a unique mixture allowing saliva to accredit all its functions [4]. Whole saliva is a rich biofluid that is gaining more attention as diagnostic source of unique biomarkers that may recognize oral and systemic diseases [5].

In diabetic patients, saliva shows multiple changes in its components as levels of glucose, proxidase, proteins and buffering ability which could help in the principle of diagnosis of diabetes [6,7]. So evaluation of salivary glucose could be a cost effective and a noninvasive method for screening, diagnosis and monitoring of diabetes [8] which has long been a focus of research and debate [9].

2. Subjects, Materials and Methods

2.1. Design and participants

This study is a comparative study, conducted in family medicine

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outpatient clinics, Cairo university hospitals, where 104 diabetic patients diagnosed according to ADA 2016 or under treatment with antihyperglycemic drugs, 50 prediabetics and 50 non-diabetic adult controls, were recruited to evaluate their glycemic state (FBS) and salivary glucose levels from January 2016 to August 2018.

From history & examination, patients who were Smokers, Alcoholics, pregnant & lactating females, previous history of surgery, radiotherapy or diseases affecting salivary glands eg Sjogren, RA, SLE.) and current medical treatment with one of these drugs (antidepressant, anxiolytic, antipsychotics, antihistaminic, clonidine and cortisone), were excluded from the study.

Ethical approval was obtained from the research & ethical committee of faculty of medicine, Cairo University. Informed written consent was designed to be used for each participant after explaining objectives and steps of the study.

2.2. Data collection tools and techniques

A previously designed questionnaire was used to interview all participants and it included Socio-demographic data (age and sex), lifestyle data (smoking and alcohol intake), medical data (diabetes duration, diseases of salivary glands, surgery, radiation, any other chronic medical condition), medication data (current treatment of diabetes, current drug untake), with oral and general examination, blood sampling using venipuncture.

Salivary Sampling, Unstimulated whole salivary samples were collected from each participant using Navazesh method. With stimulation, protein and electrolytes content of saliva are increased also pH of saliva [5], also the stimulating materials may change the pH and water content of saliva [10]. So it is more reliable to use unstimulated saliva. The collected blood and saliva were drawn into Gray topped tubes containing fluoride as glycolytic inhibitor, and gently inverted 5–8 times.

2.3. Analysis of collected blood and salivary samples

Each sample was assayed immediately or stored at −20 °C in case of delay. Before analyzing, Samples were centrifuged; blood and salivary glucose were measured by the GOD/POD (glucose oxidase-peroxidase) method using a commercially available kit supplied by Diamond, Egypt, using partially automatic analysis.

2.4. Statistical analysis

Data were statistically described in terms of mean ± standard deviation, or frequencies and percentages when appropriate. Comparison of numerical variables between the groups was done using one-way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi-square (χ^2) test was performed. Correlation between variables was done using Spearman rank correlation equation. Sensitivity and specificity were used to describe the diagnostic accuracy. The optimum cut off value for salivary glucose in diagnosing DM and prediabetes was determined using Receiver operator characteristic (ROC) analysis. *p* values less than 0.05 was considered statistically significant. All were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

3. Results

3.1. Demographic and medical data of participants

As shown in (Table 1), there was no statistically significant difference in sex distribution between the study groups, but regarding the age groups and body mass index (BMI), there were statistically significant difference as shown by ANOVA (one way) testing with P value < 0.001 and 0.38 respectively. This difference would not affect the research results as salivary glucose levels were not affected by age group or BMI as noted by Spearman rho correlation.

About ¼ of the study participants had other co-morbidities & 71.4% were in diabetic group. Hypertension represented the most common co-morbidity affecting 18.1% of all study participants with P value < 0.001. All other co-morbidities represent 11.8% among all participants with statistically insignificant P values as presented in (Fig. 1). Antihypertensive medications and vitamins were the most prescribed medications especially in diabetic patients.

Regarding diabetes treatment in diabetic group, 25% were treated with oral antihyperglycemic drugs, 51.0% were on insulin. 24% of diabetic participants were on no treatment as they were newly diagnosed during the study; they started treatment after confirming diagnosis of diabetes. Although 24% were newly diagnosed, participants with diabetes of long duration (>6 years) were nearly half of the diabetic patients.

3.2. Correlation of salivary glucose with blood glucose and other variables

As shown in (Table 2), the mean salivary glucose level was observed to be 23.40 ± 12.755 mg/dl in control group, 42.68 ± 20.830 mg/dl in prediabetic group and 59.32 ± 19.147 mg/dl in diabetic group. The mean FBS and salivary glucose were highest in diabetic group then pre-diabetic group and lowest in control group with statistically significant difference between the 3 groups

Table 1
Demographic and medical data of the study groups.

	Controls n 50 (%)	PreDM n 50 (%)	DM n 104 (%)	P value
Gender (male)	26 (52.0)	24 (48.0)	38 (36.5)	0.140
18–40 years	28 (56.0)	32 (64.0)	24 (23.1)	0.001*
40–59 years	19 (38.0)	16 (32.0)	63 (60.6)	
≥60 years	3 (6.0)	2 (4.0)	17 (16.3)	
Obesity	18 (36.0)	27 (54.0)	57 (54.8)	0.038*
Association of co-morbidities	6 (12.0)	8 (16.0)	35 (33.7)	0.005*
Current drug treatment	5 (10.0)	8 (16.0)	34 (32.7)	0.003*
Duration of diabetes > 6 years			50 (48.1)	
Treatment of diabetes				
Oral antihyperglycemic			26 (25.0)	
Insulin			48 (46.2)	
Both			5 (4.8)	
No treatment			25 (24)	

*Indicates significant P value.

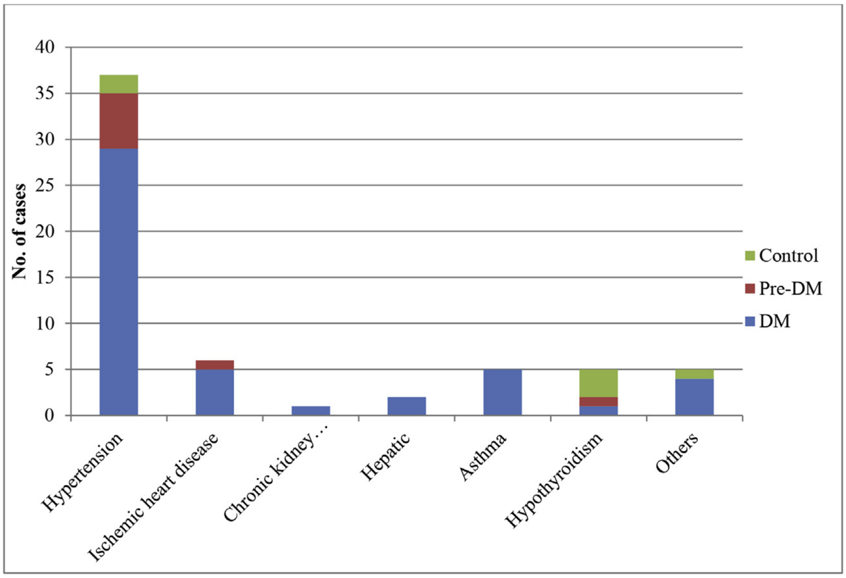

Fig. 1. Distribution of different co-morbidities among the study groups.

Table 2
FBS and salivary glucose levels of the studied groups.

	FBS (mg/dl)	Salivary glucose (mg/dl)	r	P value
Controls (n 50)			0.67	0.001*
Mean (±SD)	86.45 (±8.93)	23.40 (±12.76)		
Maximum	99	50		
Minimum	70	0		
PreDM (n 50)			0.36	0.01*
Mean (±SD)	111.31 (±6.76)	42.68 (±20.83)		
Maximum	124	80		
Minimum	100	0		
DM (n 104)			0.56	<0.001*
Mean (±SD)	226.89 (±107.85)	59.32 (±19.15)		
Maximum	607	90		
Minimum	70	10		
P Value	0.001*	0.001*		<0.001*

*Indicates significant P value.
r: Correlation Coefficient.
SD: Standard Deviation.

with P value ≈ 0.001 and ≈ 0.001 respectively.

Salivary glucose level was significantly correlated to FBS with strong positive association in non-diabetic control group & diabetic group with Correlation Coefficient r = 0.67, P value < 0.001 and r = 0.56, P value 0.001 respectively. In pre-diabetic group it was significantly correlated to FBS with moderate positive association with r = 0.36 & P value ≈ 0.01 as shown in (Table 2).

The diabetic patients with controlled diabetes mellitus represented only 31.73%, while uncontrolled diabetics were 68.27% (the patient was considered controlled if FBS was 80–130 mg/dl according to ADA 2017). In controlled diabetic patients, the mean FBS was 114.72 ± 14.50 mg/dl, and the mean salivary glucose 45.70 ± 14.91 mg/dl. While in uncontrolled diabetic patients, the mean FBS was 274.37 ± 98.75 mg/dl, and the mean salivary glucose 65.65 ± 17.60 mg/dl, With significant difference in salivary glucose levels with P value < 0.001.

Regarding correlation between salivary and FBS, within uncontrolled diabetes group, there was significant correlation with moderate positive association (r = 0.38 & P value˜0.001). While within controlled diabetes group, there was insignificant correlation (r = −0.11 & P value˜0.546) which can be attributed to the small number of participant in controlled diabetes group.

In (Table 3), it was observed that FBS and Salivary glucose were not correlated to age group, sex, BMI, co-morbidities, drug treatment, duration or treatment of diabetes with statistically insignificant P values in diabetic and non-diabetic groups. While in prediabetic group, FBS was significantly correlated to age group, presence of hypertension and antihypertensive drugs with P value˜0.02, ≈0.001 and ≈ 0.001 respectively. While salivary glucose was significantly correlated to co-morbidities and drug treatment with weak positive association (r = 0.28 and P value˜0.05).

3.3. Diagnostic accuracy of salivary glucose assessment

Based on (Table 4), salivary glucose could significantly differentiate prediabetics from non-diabetic controls with P value < 0.001. The area under the ROC curve was 0.784 (Good Accuracy). The curve showed that the optimal cut-off value for differentiating controls from prediabetics is 22.50 mg/dl with sensitivity (86%) and specificity (52%). It could differentiate non-diabetic controls from diabetics with P value < 0.001. The area under the ROC curve was 0.928 (Excellent Accuracy), revealing the optimal cut-off value as 27.00 mg/dL with sensitivity (94.23%) and specificity (62%). Salivary glucose could also differentiate prediabetics from diabetics with P value < 0.001. The area under the ROC curve was 0.718 (Good Accuracy), showing the optimal cut-off value as 42.50 mg/dL with sensitivity (76%) and specificity (52%).

4. Discussion

In our study, salivary glucose was detected in diabetic, prediabetic and non-diabetic subjects. This was in agreement with the observations of [3,9,11–13] who also found glucose in saliva of the diabetic and non-diabetic subjects. However, Amer et al. did not identify glucose in the saliva of non-diabetic subjects. This could be explained by the used test [14].

We found that the mean salivary glucose levels were significantly higher in diabetic subjects (59.32 ± 19.147 mg/dl) and in prediabetic subjects (42.68 ± 20.830 mg/dl) than non-diabetic subjects (23.40 ± 12.755 mg/dl) with P value < 0.001. This was in harmony with the study of Gupta et al. which found mean salivary

Table 3
Correlation between salivary glucose level and all variables in the study group.

	Controls		PreDM		DM	
	r	P value	r	P value	r	P value
Age group	0.23	0.11	0.16	0.28	−0.03	0.76
Sex	3.00	0.47	0.05	0.72	0.18	0.07
BMI	0.18	0.21	0.01	0.99	0.12	0.22
Co-morbidities	0.002	0.99	0.28	0.05*	0.18	0.07
HTN	0.21	0.14	−0.21	0.14	−0.18	0.07
IHD	–	–	0.11	0.45	−0.07	0.46
CKD	–	–	–	–	0.14	0.15
hepatic	–	–	–	–	0.11	0.29
asthma	–	–	–	–	0.01	0.92
hypothyroidism	−0.20	0.16	−0.23	0.10	−0.04	0.67
others	0.05	0.76	–	–	−0.16	0.09
Current drug treatment	0.08	0.56	0.28	0.05*	0.15	0.14
Antihypertensive	0.12	0.41	−0.21	0.14	−0.18	0.07
Anticoagulant	0.05	0.76	−0.02	0.89	–	–
L-troxin	−0.20	0.16	−0.23	0.10	−0.04	0.67
TTT of PN	–	–	–	–	−0.01	0.96
Vitamines	–	–	–	–	0.15	0.13
others	–	–	0.11	0.45	−0.11	0.29
FBS	0.67	0.001*	0.36	0.01*	0.56	0.001*
Duration of diabetes	–	–	–	–	0.06	0.58
Treatment of diabetes	–	–	–	–	0.03	0.76
FBS in controlled DM					−0.11	0.546
FBS in uncontrolled DM	–	–	–	–	0.38	0.001*

*Indicates significant P value r correlation coefficient.

Table 4
Diagnostic accuracy of salivary glucose assessment.

	PreDM-Controls	DM-controls	PreDM-DM
AUC	0.784	0.928	0.718
P value	0.001*	0.001*	0.001*
Cut off value	22.50	27.00	42.50
Sensitivity %	86.00	94.23	75.96
Specificity %	52.00	62.00	62.00
+ve PV	64.18	83.76	76.70
-ve PV	78.79	83.78	50.98
Accuracy	69.00	83.77	68.18
95% CI			
Lower	0.692	0.889	0.633
Upper	0.876	0.967	0.802

*Indicates significant P value.
AUC area under the curve.
CI confidence interval.
+ve PV positive predictive value.
-ve PV negative predictive value.

glucose levels (19.48 ± 5.511) in diabetics to be significantly higher than the levels in non-diabetics (7.82 ± 2.423) with P value < 0.00 [9]. and Akasapu et al. observed that the mean salivary glucose was 4.272 ± 2.23 for healthy controls and 13.603 ± 5.599 for diabetic group [15]. The study of Al-Zahawi et al. in Baghdad also found that mean salivary glucose in controlled diabetic group 17.98 ± 1.03, in uncontrolled diabetic group 15.57 ± 1.03 and 10.11 ± 1.08 in non-diabetic group with significant P value < 0.00 [16]. Many other studies showed statically significant difference between levels of salivary glucose in diabetic and non-diabetic subjects as [3,11,17–22].

As noted from previously presented results, in our study the mean salivary glucose level in all study groups is higher than recorded from other studies values. This may be attributed to many factors as;

The unstimulated Salivary samples collected in our study reflect glucose levels not only due to leakage across basement membrane salivary glands but also from gingival crevicular fluid [3] which could be blamed for increased glucose levels [21]. Other studies used the parotid saliva only as in the study of Andersson et al. [23], others collected the saliva with “spit technique” where the participant had to spit in a sterile cup every minute for 10 min as in the studies of Balan et al. [3] and Kumar et al. [19] which could be a kind of stimulation of the salivary glands by movement.

In the study of Takeda et al., they have measured salivary chemical concentrations under variable situations in a healthy subjects, it was found that almost all metabolites were higher in unstimulated saliva compared to stimulated saliva [24]. This was also observed by Jha et al. who found the mean salivary glucose levels higher in unstimulated saliva of both controlled and uncontrolled diabetics compared to stimulated saliva [20].

According to López et al., persistent hyperglycemia leads to microvascular changes in the blood vessels of salivary glands and its basement membrane. This leads to increased leakage of glucose from the ductal cells. Thus salivary glucose is also influenced by extent of salivary gland damage by diabetes which is unpredictable [25]. In our study, participants with diabetes of long duration (>6 years) were nearly half (48.1%) of the diabetic patients and only 31.73% were controlled, which suggest that our diabetic group participants were more prone to the long term effects of diabetes on salivary glands.

Also the difference in method used to estimate salivary glucose, [glucose oxidase method (GOD-POD)] used in our study with enzymatic colorimetric test kit, GOD-PAP in other studies like that of Amer et al. [14]. Also the different study populations with different eating patterns may contribute as explained by Balan et al. [3].

Regarding correlation between salivary and blood glucose levels, it was observed that salivary glucose levels increased with the increase in serum glucose levels in diabetic, pre-diabetic and nondiabetic subjects with significant correlation coefficient (r) and strong positive association ($r = 0.56$ & P value < 0.001in diabetics, $r = 0.36$ & P value 0.01in prediabetics, $r = 0.67$ & P value < 0.001 in nondiabetic controls). These findings were in consistence with those of [3,9,11,19,21]. The correlation coefficient was $r = 0.30$ in non-diabetics and $r = 0.67$ in diabetics as announced in a meta-analysis studying the universal correlation [26].

But Azizi & Modaberi [27] and Hedge et al. [28] found a significant correlation only in diabetic subjects. This difference from our results may be attributed to the fact that salivary glucose changes in very small range in healthy individuals.

In the current study, in controlled diabetic patients, the mean salivary glucose was 44.79 ± 14.61 mg/dl. In uncontrolled diabetic patients, the mean salivary glucose was 66.07 ± 17.58 mg/dl. These levels showed statistically significant difference with P value < 0.001. As also revealed in studies conducted by Refs. [3,19,20,22], where the uncontrolled diabetics had higher mean salivary glucose level than the controlled diabetics with highly statistically significant. In contrast with the findings observed by Al-Zahawi et al., where the mean salivary glucose levels were higher in the controlled (17.98 ± 1.03) than uncontrolled diabetic group (15.57 ± 1.03) with statistically insignificant difference (p value 0.12) [16].

Within uncontrolled diabetics, there was significant correlation with FBS with moderate positive association ($r = 0.38$ & P value 0.001). While within controlled diabetics, there was insignificant correlation with weak association ($r = -0.11$ & P value 0.546) which can be attributed to the small number of participant with controlled diabetes in our study. The study of Mussavira et al. recorded that salivary glucose showed strong positive correlation with FBS (all diabetic patients: $r = 0.9$, $P < 0.001$; controlled cases: $r = 0.9$, $P < 0.001$; uncontrolled cases: $r = 0.922$, $P < 0.001$) [17]. Also the systematic review conducted by Mascarenhas et al. reported that the strength of the correlation between salivary and blood glucose increases with higher glycemia [26].

We observed that salivary glucose could significantly differentiate non-diabetic controls from diabetics with P value < 0.001. The area under the ROC curve was 0.928 (Excellent Accuracy). The curve showed that the optimal cut-off value for differentiating non-diabetics from diabetics is 27.00 mg/dL with sensitivity (94.23%) and specificity (62%). In the study conducted by Naik et al., they achieved a high sensitivity of 99.25% and specificity of 61.73% [11]. and the study of Kumar et al. concluded that the sensitivity was 83.33% and specificity 100% [19].

Salivary glucose could significantly differentiate prediabetics from non-diabetic controls with P value < 0.001. The area under the ROC curve was 0.784 (Good Accuracy). The curve showed that the optimal cut-off value as 22.50 mg/dL with sensitivity (86%) and specificity (52%). Also could significantly differentiate prediabetics from diabetics with P value < 0.001. The area under the ROC curve was 0.718 (Good Accuracy), with optimal cut-off value as 42.50 mg/dL with sensitivity (76%) and specificity (52%).

For differentiating diabetic subjects from nondiabetic controls, the predictive value of a positive test in this study was 83.76%. And the predictive value of a negative test in this study was 83.78% which was in harmony with the study of Naik et al. [11], where the predictive value of a positive test was 75.28%, while the predictive value of a negative test in the study was 98.6%.

In the current study, we were trying to determine the linear regression analysis between the blood and salivary glucose levels among the diabetic, prediabetic and non-diabetic control group. A linear regression equation to determine FBS if salivary glucose is known was devised as;

For non-diabetic controls, $FBS = 76.548 + (0.423 \times \text{salivary glucose})$, for prediabetics, $FBS = 106.35 + (0.116 \times \text{salivary glucose})$, and for diabetics, $FBS = 51.064 + (2.964 \times \text{salivary glucose})$. Also in studies of Gupta et al. [9] and Arora et al. [29], they were able to derive a linear regression equations.

5. Conclusion

Salivary glucose level was significantly correlated to FBS with strong positive association in non-diabetic control group and diabetic group, and with medium positive association in prediabetic group. The high sensitivity achieved indicates that saliva can serve as a good screening tool in diagnosis of diabetes and prediabetes. And the good specificity indicates that saliva can offer an adjunct diagnosis tool.

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