ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Association Of Salivary Glucose, Salivary Amylase, Serum Amylase And Serum Glucose In Diabetes And Prediabetes.

Anil Kumar Munta[1], Vijaya Radhika Pyda[2], Naga Sai Naveen Vattam[3], and Lakshmi Keerthana Badam*.

[1]Associate Professor, Department of Biochemistry, Madhubani Medical College, Madhubani, Bihar – 847212, ORCID: 0000-0002-9628-8488,
[2]Assistant Professor, Department of Biochemistry, Rangaraya Medical College(RMC), Kakinada, Andhra Pradesh-533001.
[3]Intern, Maharajah's Institute of Medical Sciences, (MIMS), Nellimarla, Vizianagaram, Andhra Pradesh – 535217.
*Assistant Professor, Department of Biochemistry, GSL Medical College, Rajahnagaram, Andhra Pradesh- 533296, India.

ABSTRACT

Diagnosis, monitoring of global epidemic diabetes rests on invasive blood glucose estimation procedures There is a critical need for development of noninvasive procedure for diagnosing and monitoring Diabetes. This study aims to compare and correlate salivary glucose, salivary amylase, serum glucose, serum amylase in pre diabetes, diabetes and controls. 137 Subjects included in this comparative cross-sectional study are divided into three groups; Group 1 (50 Type 2 diabetes), Group 2 (42 Prediabetes) and Group 3 (45 Controls). Glucose, Amylase were estimated in serum and saliva of study population. ANOVA and post hoc test were used determine the p values. Pearsons r value is used to assess the correlation between parameters. Salivary glucose and amylase are significantly elevated in group 1, group 2 compared to group 3. Serum amylase is significantly decreased in group 1, group 2. Salivary glucose is correlated to serum glucose (diabetes +0.538, prediabetes + 0.415,). Salivary amylase shows correlation with serum glucose (diabetes +0.431, pre diabetes +0.725). Serum amylase is negatively associated to serum glucose in diabetes(-0.517), prediabetes(- 0.328). Salivary glucose, salivary amylase are increased in diabetes , prediabetes and have potential role as markers in their noninvasive monitoring.

Keywords: Salivary glucose, Serum glucose, Prediabetes, Salivary amylase, Diabetes.

https://doi.org/10.33887/rjpbcs/2021.12.1.4

Corresponding author

ISSN: 0975-8585

BACKGROUND

Diabetes mellitus (DM) is one of the most common global disease, that impairs primarily glucose metabolism. It was reported for the first time about 3000 years ago in the ancient Egyptian literatue[1]. It presents mainly with glucose intolerance due to insulin inefficiency or insufficency or both[2]. The epidemic of type 2 Diabetes mellitus has become a common global health burden and accordingly a recent survey by International Diabetes Federation shows around 9.3% of adults (463million individuals) are suffering from this disease The main concern is that this figure will rise further to 578 millionby 2030.[3] The number is increasing epidemically as a consequence of an aging population and changes in lifestyle.[4] Millions of the undiagnosed individuals at present, an enormous crowd with diabetes are rolling in the direction of complications unprepared. Prevention, well-timed diagnosis and management are important in patients with DM.[5] For this reason, the investigative procedures must be customarily employed to diagnose and monitor diabetes. The analysis of blood glucose is the most consistently employed procedure. However, the invasive procedures offer a significant degree of discomfort and anxiety, especially to pediatric and geriatric patients. Considering this, there is a critical need for the development of noninvasive procedure for diagnosing and monitoring diabetes.[6]

The serum amylase level is decreased due to exocrine dysfunction in DM type II. Serum amylase can be an additional informative parameter for the assessment of severity and progression of the dreadly disease as well as its response to ongoing therapy.[18] Saliva plays an important role in maintaining the equilibrium of the oral cavity and is considered as “mirror of the body” as it represents the general health status. Moreover, collection of saliva is far easier due to its accessibility and availability.[8] The markers present in saliva and serum will be useful in the diagnosis of a variety of systemic disorders. The analysis of saliva can offer a cost-effective approach for screening of diabetes and may represent an alternative for the individuals in whom the blood drawing is complicated. Numerous studies, like Streckfus et al.,[7] Indira et al.,[8] (salivary glucose , salivary amylase, total proteins), Nagalakshmi et al.,[6] Ficara et al.,[13] (gingival fluid glucose, protein), Panda et al.,[14] (salivary glucose), Panchbhai et al., [15] Raghunathan et al.,[17] Jain et al.,[18] had been done to uncover the diagnostic role of non invasive salivary parameters in diabetes.

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are synonyms and depends on the test used to detect the diabetes. IFG refers to a condition where the fasting blood glucose is increased above the normal levels but not high enough to be considered as diabetes mellitus. This prediabetes state, is associated with insulin resistance and other metabolic impairemnts, although of lesser risk than diabetes mellitus. [11] As per the criteria of the American Diabetes Association (ADA), persons with the fasting blood sugar of 100 to 125 mg/dL, post prandial blood glucose of 140 to 199 mg/dL are diagnosed with prediabetes. [12]

MATERIALS AND METHODS

The subjects enrolled in this study are from those attending the regular outpatient department of Maharajah’s Institute of Medical Sciences. The present study was conducted on a total of 137 subjects of 40-70 years age group. The study population was divided into three groups; Group 1 (fifty type II Diabetes patients), Group 2 (forty two prediabetes patients) and Group 3 (forty five healthy individuals as control). During the study period, informed written consent was taken and detailed case history was obtained by providing questionnaire to each individual.

Ethical Authorisation of the study

This was a comparative cross sectional study and the study protocol was approved by the Institutional Ethics Committee from the Maharajah’s Institute of Medical Sciences affiliated to Dr. NTR University of Health Sciences. (Number: 2019/01 Date: July 13, 2019).

Inclusion criteria

Patients are diagnosed with diabetes mellitus (based on ADA critera)[12] are included in group I. Group II included patients diagnosed with pre diabetes and group III included non diabetic individuals.

Exclusion criteria

The subjects with smoking, alcoholism, pregnancy, hypertension, thyroid disorders, mumps, renal impairment, liver diseases, and pancreatic disorders were excluded as these factors influence the parameter values.

Blood sample collection

Age and sex of participants were noted, detailed history was taken and clinical examination was performed. 2 ml. of blood after an overnight fast was collected from the respective groups (diabetes, prediabetes and control) under aseptic conditions in red vaccutainer tubes and the clotted samples were centrifuged. The serum was separated and used for analysis of glucose and amylase.

Saliva sample collection

The participants were detailed about the saliva sample collection. The intake of food and liquids were resticted for two hours before the sampling. All the saliva samples were collected in the fasting state from 8 AM to 11 AM to avoid the diurnal variation. After washing the mouth with distilled water, the unstimulated saliva was collected in a sterile plastic container for a period of five minutes from the study groups. The collected saliva samples were immediately analysed.

METHODS

The analysis of serum glucose was performed by glucose oxidase and peroxidase (GOD-POD) method on autoanalyser (14). The determination of serum amylase levels was performed by 2-Chloro-4-Nitrophenyl-β-1-4galactopyranosylmaltotrioside (CNP-G) method (kinetic assay) on auto analyser (15). The rate of 2-chloro-4-nitrophenol formation is monitored at 415 nm and is proportional to alpha amylase activity in serum.

By placing saliva in sample cups of autoanalyzer, salivary glucose is estimated using GOD-POD method. Salivary amylase is estimated after diluting the saliva by taking 10µL of saliva and mixing it with 990 µL of distilled water(1 in 100 dilution), the diluted saliva samples are placed in sample cups and analysed in autoanalyzer using CNP-G method amylase kits.

Statistical Analysis

The data obtained was analysed statistically using SPSS (Statistical Package for the Social sciences) software version 17. The mean and standardard deviation were calculated for each parameter. ANOVA and Tukey's test for post-hoc analysis were applied for the comparison of variables in all three groups. The p value <0.05 was considered as statistical significant. Pearson correlation coefficient was calculated for salivary glucose and serum glucose, salivary amylase and serum glucose, serum amylase and serum glucose to findout their degree of correlation with serum glucose.

RESULTS

Total 137 subjects were included and investigated in the current study. Group 1 includes 50 subjects (31 males and 19 females) of diabetes, group 2 includes 42 subjects (30 males and 12 females) of prediabetes and group 3 includes 45 non diabetic participants (23 males and 22 females). The age group (years) ranges from 40-70 years in all the 3 groups with a mean of 59.86 ± 4.6 in Group 1, 51.22 ± 10.0 years in Group 2 and 41.34/ ± 2.7 years in Group 3 subjects. (Table-1).

The mean and standard deviation, F-ratio and p-values of serum fasting and salivary glucose, serum and salivary amylase levels between diabetes and prediabetes groups showed significant difference in comparison to the values of control group ($p <0.05$) (Table 2). The mean serum glucose value in diabetics was 204 ± 12.76 mg/dl and the mean salivary glucose was 3.8± 0.7/mg/dl. In prediabetes the mean serum glucose level was 111.65 ± 6.91 mg/dl while mean salivary glucose level was found to be 2.15 ± 0.89 mg/dl. In non diabetics the mean serum glucose level was 84.25 ± 7.02 mg/dl and mean salivary glucose level was found to

ISSN: 0975-8585

be 0.7 ± 0.16 mg/dl. All the study groups registered significant differences between serum and salivary glucose levels ($p < 0.05$) (Table 3), (Figure 1).

In the DM patients of group 1, the mean value of serum amylase was 50.65 ± 8.50 U/L, which is lower when compared to other groups and mean salivary amylase was 232.47 ± 20.25 U/L. In pre diabetes participants of group 2 the mean serum amylase level was 58.31 ± 8.44 U/L and the mean salivary amylase level was 221.27 ± 4.63 U/L. In non diabetes participants of group 3 the mean serum amylase level was 71.86 ±10.00 U/L which is higher among the 3 groups and the mean salivary amylase level was 143.06 ± 15.43 U/L, that is lower in comparison to other groups. The serum and salivary amylase levels between Group 1, Group 2 and Group 3 shows significant differences ($p < 0.05$) (Table 4).

Table 1: Age and gender distribution of the study Groups.

Parameters	Group I (Diabetics)	Group 2 (Group 2Prediabetics)	Group 3 (Control)
Male	31(62%)	28 (66.6%)	23 (51.1%)
Female	38 (38%)	14 (33.3%)	22 (48.8%)
M:F ratio	1:63	2.3	1.04
Age ± SD	59.86 ± 4.6	51.22 ± 10.05	41.34 ± 2.74

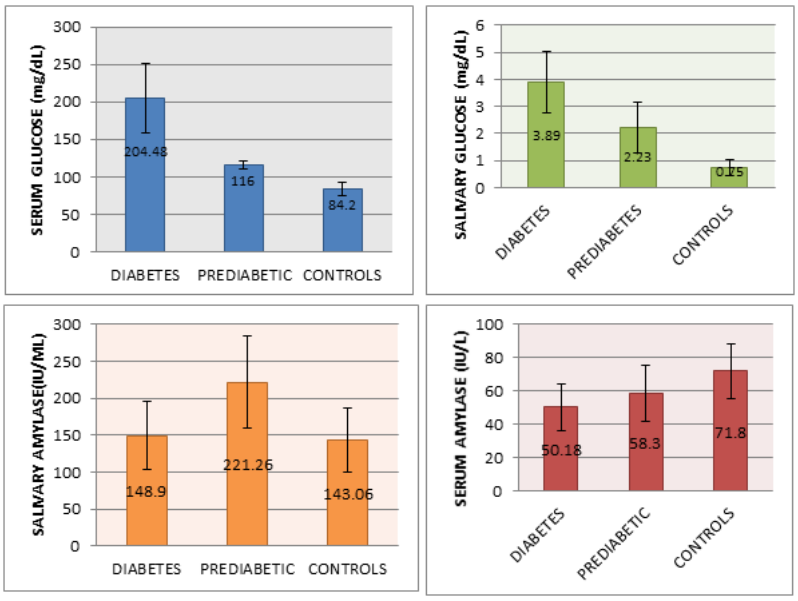
SD-Standard Deviation, M:F- Male Female ratio

Table 2: Comparison of mean of variables in the study population

Parameter	Control	Prediabetes	Diabetes	F Value	P
Serum glucose (mg/dl)	84.25±7.02	111.65± 6.91	204± 12.76	2032.5	<0.05
Salivary glucose (mg/dl)	0.72 ± 0.16	2.15± 0.89	3.81± 0.79	229.21	<0.05
Serum amylase (U/L)	71.86±10.00	58.31± 8.44	50.65±8.50	62.47	<0.05
Salivary amylase (U/L)	143.06±15.43	221.27± 4.63	232.47±20.2	467.58	<0.05

(p value < 0.05 significant), F= One-way ANOVA

Fig 1: Bar diagrams depicting mean concentration of analytes in the three groups.



ISSN: 0975-8585

Table 3: Comparison of mean serum FBS, salivary glucose , serum amylase, salivary amylase in all the study groups with Tukey's post-hoc test

	Serum glucose		Salivary glucose		Salivary amylase		Serum amylase	
	Group 1 vs Group 3	Group 2 Vs Group 3	Group 1 vs Group 3	Group 2 Vs Group 3	Group 1 vs Group 3	Group 2 Vs Group 3	Group 1 vs Group 3	Group 2 Vs Group 3
p value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 4: Comparison of Pearson correlation coefficient (r value) of salivary glucose, salivary amylase, Serum Amylase to Serum glucose in all groups.

Serum glucose in	Salivary Glucose		Salivary amylase		Serum amylase	
	r value	p value	r value	p value	r value	p value
Diabetes	+ 0.538	<0.05	+0.431	<0.05	-0.517	<0.05
Pre diabetes	+0.415	<0.05	+0.725	<0.05	-0.328	<0.05
Controls	+0.687	<0.05	+0.619	<0.05	-0.192	0.17

DISCUSSION

Diabetes mellitus is a group of metabolic diseases known from ancient times. This is one of the major causes for morbidity and mortality. It is characterized by relative or absolute insulin deficiency, insulin resistance or both and is primarily manifested as hyperglycemia. The effect of insulin dysfunction shows its effect on carbohydrate, lipid and amino acid metabolism.(8). The resultant longterm hyperglycemia , contributes to microvascular and macrovascular complications in diabetes causing significant morbidity and mortality in this population. This study was undertaken for evaluation of potential role of salivary biomarkers in non invasive diagnosis and monitoring in diabetes and prediabetes.

This study showed that the serum and salivary glucose levels were higher in diabetes (Group 1) and in prediabetes (Group 2) when compared to controls (Group 3) and the difference was statistically significant ($p<0.05$). Unstimulated whole saliva was used for the analysis of salivary parameters. The current study registered significant decrease in mean salivary amylase levels in diabetics when compared to prediabetics and healthy individuals. The salivary glucose was found to be markedly elevated in diabetes and our study is in corroborating with the studies of Harrison et al., [10] and Panchbahai et al., [15] However, Ficara et al., [13] and Marcheti et al., [16] reported no significant elevation of salivary glucose among the diabetics and controls. The salivary glucose values are significatantly elevated in prediabetes group when compared with controls similar to study of Fares et al.,[26] Hyperglycemia results in the elevation of salivary glucose. This is because of diabetic membranopathy, which leads to leakage of glucose across the basement membrane and raised glucose percolation to saliva from circulation. Frequent monitoring of serum glucose level is required to reduce the diabetes complications.Their is significant positive correlation of salivary glucose with serum glucose in all three groups (diabetes r value +0.538 , prediabetes +0.415, controls +0.687) similar to study of Fares et al.[26] The outcome of present study showed significant differences among salivary glucose concentrations of normal, prediabetes and diabetes patients suggesting that monitoring of salivary glucose level can be used as potential marker as an index of diabetes mellitus. [18].

The present study shows significant difference among salivary amylase levels in three groups with salivary amylase high in diabetes compared to controls similar to studies of Indira et al., [8] Panchbahai et al., [15] The salivary amylase is also significantly increased in Prediabetes. The elevated salivary amylase activity was attributed to altered taste sensations in Diabetes by Dods et al and due to stress by Yavuzyilmaz et al., and Chatterton RT et al., but the study of Prathibha et al., [25] shows significant decrease in salivary amylase levels in diabetes. Our study showed marked increase in salivary amylase in prediabetes compared to healthy individuals similar to the studies of Malathi L et al.,[24]. The salivary amylase is positively correlated with serum glucose in diabetes (r value +0.431 and prediabetes (r value +0.725) highlighting the potential role of salivary amylase in diagnosis and monitoring of diabetes and prediabetes.

The current study reports that the mean serum amylase level was significantly decreased in diabetes and pre diabetes individuals in comparison to control. This is in agreement with results of Ata et al., [19] , Aughsteen et al., [20]. On the contrary, Farhood et al., [21] Abdelsalam et al., [22] demonstrated that the serum amylase levels were significantly elevated in type 2 diabetic subjects. The occurance of lower serum amylase in diabetes mellitus may be associated with the derangement of exocrine-endocrine axis. The decreased levels of serum amylase can be seen in endocrine disorders like diabetes due to impaired islet β cell function. Serum amylase is negatively correlated to serum glucose levels in diabetes (r value -0.517) and prediabetes (r value -0.328)

Overall, both salivary glucose and salivary amylase show good potential to discriminate the patients with diabetes from the non diabetes. The outcome of the present study shows a significant difference in salivary parameters between normal, prediabetes and diabetes patients suggesting that monitoring of salivary parameters can be potentially used as an index of onset of diabetes mellitus.

Serum amylase in our study shows significant difference among three groups and is negatively correlated to serum glucose in diabetes and prediabetes but further studies have to be undertaken to elaborate its role as marker in management of prediabetes and diabetes.

However, because of certain limitations in this present study, the values should be correlated with other confounders like duration of diabetes, oral hygiene, life style, age etc. Further studies on large sample size may evaluate the diagnostic power of salivary parameters in Diabetes and Prediabetes. In diabetes, the extent of end organ damage may be assessed in a noninvasive manner using salivary glucose and amylase if longitudinal studies can be undertaken in this context.

CONCLUSION

The significant difference in values of salivary glucose and amylase among diabetes, prediabetes, control groups and their significant correlation to serum glucose in present study can be used as basis for employing these parameters in diagnosis and monitoring of prediabetes and diabetes. Further studies on large sample size should be done to ascertain the diagnostic value of salivary glucose and salivary amylase in prediabetes and diabetes

ACKNOWLEDGEMENT

We are indebted to the MIMS for funding of this study and cooperation extended to us, without which this work would not be possible. We are also grateful to the study group for their cooperation.

REFERENCES

[1] Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends.*Oman Med J* 2012;27: 269–73. doi: 10.5001/omj.2012.68

[2] Piero M. Diabetes mellitus – A devastating metabolic disorder. *Asian J Biomed Pharm Sci* 2015;4:1–7. doi: 10.15272/ajbps.v4i40.645

[3] Pouya saeedi, Inga Peterson, Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the international diabetes Federation Diabetes Atlas 9 th edition.

[4] Singh S. The genetics of type 2 diabetes mellitus: *A review. J Sci Res* 2011;55:35–48. doi: org/10.1373/clinchem.2010.157016

[5] Patel P, Macerollo A. Diabetes mellitus: Diagnosis and screening. Am Fam Physician. 2010;81: 863–70.

[6] Nagalaxmi V, Priyanka V. Can saliva be a marker for predicting type 1 diabetes mellitus.-A pilot study? *J Indian Acad Oral Med Radiol* 2011;23: 579–82. doi: 10.4103/jomfp.JOMFP_15_20

[7] Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. *Oral Dis* 2002;8: 69–76. doi: 10.1034/j.1601-0825.2002.1o834.x

[8] Indira M, Chandrashekar P, Kattappagari KK, Chandra LK, Chitturi RT, Ramana Reddy BV. Evaluation of salivary glucose, amylase and total protein in type 2 diabetes mellitus patients. *Indian J Dent Res* 2015; 26: 271-75. doi: 10.4103/0970-9290.162883.

[9] López ME, Colloca ME, Páez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. Braz Dent J. 2003;14(1):26-31. doi: 10.1590/s0103-64402003000100005. Epub 2003 Jul 31. PMID: 12656461.

[10] Harrison R, Bowen WH. Flow rate and organic constituents of whole saliva in insulin dependent diabetic children and adolescents. *Pediat Dent* 1987; 9(4): 287-91.

[11] Nichols GA, Hillier TA, Brown JB. Progression from newly acquired impaired fasting glucose to type 2 diabetes. *Diabetes Care* 2007; 30 (2): 228–33. doi: 10.2337/dc06-1392.

[12] American Diabetes Association "2 .Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabete , Diabetes Care.2019; 42 : Supplement 1: 13–28.

[13] Ficara M, Levin MP, Grower MF, Cramer GD. A comparison of the glucose and protein content of gingival fluid from diabetic and nondiabetics. *J Period Res* 1975; 10(3): 171-75. doi: 10.7860/JCDR/2016/19135.799

[14] Panda A, Venkatapathy R, Nirima O. Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes Metab Syndr Obes*. 2012; 5: 149–154. doi: 10.2147/DMSO.S32112

[15] Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. *J Oral Sci* 2010;52(3): 359-68. doi: 10.7860/JCDR/2014/8239.5042

[16] Marcheti P, Tognarelli M, Giannarelli R, Grossi C, Picaro L, di Carlo A et al Decreased salivary glucose secretory rate; usefulness for detection of diabetic patients with autonomic neuropathy. *Diab Res Clin Prac* 2001; 7 (3): 181-86. doi: 10.7860/jcdr/2016/19135.7995

[17] Raghunathan H, Aswath N, Sarumathi T. Salivary glucose estimation: A noninvasive method. *Indian J Dent Sci* 2019; 11(1) : 25-27. doi: 10.4103/IJDS.IJDS_78_18

[18] Jain R, Jain PK, Mangukiya K. Study of serum amylase in the patients of type 2 diabetes mellitus. *Int J Sci and Nat* 2014; 5(3): 553-6. doi: 10.3329/jbsp.v12i2.35422

[19] Ata N, Dal K, Kucukazman M, Yeniova AO, Karakaya S, Unsal O. The effect of glycemic control on CEA, CA 19-9, amylase and lipase levels. *Open Med* 2015; 10: 8-13.doi:10.1515/med-2015-0002

[20] Aughsteen AA, Abu-Umair MS, Mahmoud SA. Biochemical analysis of serum pancreatic amylase and lipase enzymes in patients with type 1 and type 2 diabetes mellitus. *Saudi Med J* 2004; 26(1): 73-7.

[21] Farhood HB, Al-salih RMH, Radhi MN. Clinical studies to evaluate pancreatic functions in the patients of type 2 diabetes mellitus. *Int J Innov Appl Sci* 2014; 7(1): 413-20.

[22] Abdelsalam KEA. Lipoprotein lipase, amylase and triglyceride alteration: lean diabetic and obese (nondiabetic) patients. *Int J Basic Appl Res* 2015; 23(2): 95-103. doi : 10.7860/JCDR/2013/6016.3120

[23] Raphael EG, Engracia TS, Chastene CD, Flake AR. Estimation of salivary glucose, amylase, calcium, and phosphorus among non-diabetics and diabetics: Potential identification of non-invasive diagnostic markers.. *Dia Met Syn Clin Res Rev* 2019;13(4); 2601-2605. doi: 10.1016/j.dsx.2019.07.037.

[24] Malathi L, Masthan KM, Balachander N, Babu NA, Rajesh E. Estimation of salivary amylase in diabetic patients and saliva as a diagnostic tool in early diabetic patients. *J Clin Diagn Res*. 2013;7(11):2634-2636. doi:10.7860/JCDR/2013/7574.3634

[25] K M P, Johnson P, Ganesh M, Subhashini AS. Evaluation of Salivary Profile among Adult Type 2 Diabetes Mellitus Patients in South India. *J Clin Diagn Res*. 2013;7(8):1592-1595. doi:10.7860/JCDR/2013/5749.3232

[26] Fares S, Said MSM, Ibrahim W, Amin TT, Saad NES. Accuracy of salivary glucose assessment in diagnosis of diabetes and prediabestes. Diabetes Metab Syndr. 2019 Mar-Apr;13(2):1543-1547. doi: 10.1016/j.dsx.2019.03.010. Epub 2019 Mar 8. PMID: 31336519.