

Validation of salivary glucose as a screening tool of diabetes mellitus

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Background: Improved prognosis and delay of clinical complications in diabetes mellitus can be ensured by early screening and regular monitoring after diagnosis. This requires venipuncture at regular intervals of time causing anxiety and discomfort to the patient. Hence, development of a painless, non-invasive procedure is desirable for which saliva is a potential tool. Also, this would provide easy and cost-effective means for large scale screening and epidemiological intervention.

Aim: To measure fasting plasma glucose (FPG) and compare and correlate with salivary glucose levels in normal and diabetic individuals. Also, the correlation between glycated hemoglobin (HbA1c) and salivary glucose is studied in the diabetics and controls.

Methods: Blood and salivary glucose was estimated by GOD-POD method and glycated hemoglobin by HPLC. Statistical analysis was done on SPSS 16. Mean, Standard deviation, independent t test, ANOVA (f test), Pearson's correlation coefficient along with regression analysis was carried out and comparison was done between the control and diabetic groups and the different subgroups within the diabetic group.

Results: A significant difference between the salivary glucose levels in subjects indicating that a deranged glycemic status is reflected in saliva. Also, salivary glucose increases in proportion to an increase in the FPG and HbA1C of the diabetics. The regression coefficient was calculated and a formula was derived for prediction of FPG and HbA1c using salivary glucose.

Conclusion: Saliva can be used as a screening tool for diabetes. Standardization of the technique and setting up a reference range will also make it useful in diagnosing diabetes mellitus.

Key words: diabetes mellitus, saliva, biomarkers, mass screening, glycemic control.

INTRODUCTION

Diabetes mellitus is a silent epidemic that is massive and growing at a steady rate. The International Diabetes Federation has estimated that 10% of the global health expenditure (i.e. USD 640 billion) is spent on diagnosis and management of diabetes and there would be an approximate burden of 642 million diabetics on health services of all the parts of the world by 2040.[1]

Diabetes mellitus, a complex metabolic disease, is characterized by absolute or relative insufficiency combined with resistance to metabolic actions of insulin. It is associated with long term complications of the eyes, kidneys, heart and other organ systems of the body.[2]

Earlier, it was documented and hypothesized that diabetes mellitus is a problem

associated with the urban population of the developed countries. However, prevalence studies in the recent years suggest an alarming rise of persons having diabetes in the middle- and low-income countries with India having the second highest number of cases after People's republic of China. Also, there is a startling increase in the number of individuals being diagnosed with diabetes mellitus from the rural population.[3]

However, improved prognosis and delay of clinical complications can be ensured by early screening of type 2 diabetes mellitus with the glycemic status of the individual being regularly monitored after diagnosis. This requires sample collection by venipuncture at regular intervals of time which causes anxiety and discomfort to the patient. Also, the anxiety and mental trauma associated with venipuncture discourages the patient further.[4]

This highlights the importance of the development of a painless, non-invasive procedure that would make monitoring of glycemic status more acceptable to the patient.

Studies are being conducted to develop saliva as a tool for the screening and monitoring of chronic systemic diseases like diabetes mellitus. Being one of the most abundant secretions of the body with its easy and non-invasive collection; saliva is a potential screening tool.[5]

Also this would provide an easy and cost effective means for large scale screening and epidemiological intervention.

This study is a step towards validating saliva as a tool for screening and monitoring glycemic status of an individual.

Aim of the study

The aim of the present study is to measure fasting plasma glucose (FPG) and compare and correlate it with its salivary glucose levels in normal and type 2 diabetes mellitus patients. Also, the correlation between glycated hemoglobin (HbA1c) and salivary glucose is also studied in the persons with type 2 diabetes as well as controls.

MATERIALS AND METHODS

Institutional ethics committee gave permission to carry out the study [Ref NO:SU/2018/122]. Also, written consent was taken from each individual participating in the study.

The study comprised 200 participants between the ages of 35–70 years. The participants included 100 diabetic subjects attending the Department of Medicine Out Patient Clinic with a history of diabetes for at least 3 years, and 100 age and gender matched control subjects. The participants were recruited after the screening of 315 patients. Out of these, 115 patients were not included in the study as they came under the exclusion criteria. Patients diagnosed with type 1 diabetes mellitus, salivary gland disorders, having pan masala, beetle and tobacco chewing habits were excluded from the study. The patients were examined by the consultant in the Department of Medicine Out Patient Clinic to rule out the presence of the afore mentioned criteria.

Collection of sample

a. 4 mL of venous blood sample collected in the morning on an empty stomach after at least 8 hours of fasting. The patient was not advised to stop taking any medication the night before giving the sample. Out of the 4 mL, 2 mL of the blood were collected in the sodium fluoride-potassium oxalate (grey) vacutainers for glucose estimation and 2 mL in EDTA (lavender) vacutainers for HbA1c estimation.

b. Saliva: Saliva sample was collected at the same time, once the blood sample was collected. Subjects were asked to rinse their mouth twice with distilled water after which saliva was collected by passing drooling. Stimulated saliva has been shown to have an altered pH, water content, protein and electrolytes. [6,7] The pooled unstimulated saliva in the mouth was collected in a sterile, disposable plastic container over a period of 5 minutes. The amount of saliva secreted and collected is different in the subjects. However, it has been documented that the amount of sample collected does not affect the concentration of different analytes e.g. glucose. [6,7] The samples were stored on ice and sent to laboratory to be processed immediately. The saliva samples were then centrifuged at 2000 rpm for 5 minutes after which the supernatant was used for glucose estimation.

Blood and salivary glucose were estimated by GOD-POD method in the Dirui-300B auto analyser.[8] Glycated hemoglobin was estimated by HPLC on Bio rad D10.[9]

Statistical analysis

The obtained data were statistically analyzed by SPSS 16. Mean, Standard deviation, independent t test, ANOVA (f test), Pearson's correlation coefficient along with regression analysis were carried out and comparison was done between the control and diabetic groups and the different subgroups within the diabetic group. A p value <0.05 and <0.01 was considered significant and highly significant respectively.

RESULTS

The study comprised 200 participants (100 in each; patient and control group) between the age groups of 35–70 years. The average age of the participants was 55.2 and 54.3 years in the patient and control group respectively. The study

included 67 males and 33 females in the patient group and 65 males 35 females in the control group.

The fasting plasma glucose, salivary glucose and HbA1c levels were analyzed in all the participants of the study as shown in Table 1.

Table 1

Comparison of fasting plasma glucose (FPG), salivary glucose and HbA1c in patients and control group

Group		FPG (mg/dL)	Salivary glucose (mg/dL)	HbA1c (%)
Patients	Mean	183.36	4.37	7.7
	Standard deviation	51.53	3.59	1.73
	Minimum	101	0.6	5.3
	Maximum	311	12.8	12.6
Control	Mean	79.6	0.92	5.1
	Standard deviation	13.07	0.22	0.35
	Minimum	60	0.7	4.3
	Maximum	109	1.8	5.8
p value (on comparison between the patient and control group)		p < 0.01*	p < 0.01*	p < 0.01*

* highly significant

Independent t test was performed between the control and patient groups comparing their respective FPG, salivary glucose and HbA1c levels and the difference was highly significant with a p value <0.01.

Table 2 shows the division of patient group into 3 subgroups based on their FPG. The first group having FPG less than 126 mg/dL (as 126 mg/dL is the cut off for diagnosis of diabetes

based on FPG), second group having FPG 126–180 mg/dL (as 180 mg/dL is the renal threshold of glucose) and the third having FPG greater than 180 mg/dL (i.e just above the renal threshold of glucose – 180 mg/dL). ANOVA (f test) was used to compare the salivary glucose levels of these subgroups and the difference was found to be statistically highly significant (f value 226.4, p <0.01).

Table 2

Average levels of salivary glucose for different levels of fasting plasma glucose (FPG) in patients

FPG Groups	N	Salivary glucose (mg/dL)		f value
		Mean	Standard deviation	
Below 130 mg/dL	24	0.95	0.35	226.4** p <0.01
130–200 mg/dL	35	2.21	1.39	
Above 200 mg/dL	41	8.22	1.97	
Total	100			

** f value indicates statistically significant difference of salivary glucose for different FPG levels.

Multiple comparisons of salivary glucose were also made between different groups of FPG

in patients. All the intergroup comparisons were found to be significant as shown in Table 3.

Table 4 shows the average levels of salivary glucose of subgroups having different levels of HbA1C. The salivary glucose of these subgroups was compared using f test and the difference was found to be statistically significant.

Multiple comparisons of salivary glucose were made between different subgroups of HbA1C in patients and these intergroup comparisons were found to be statistically significant (Table 5).

Table 3

Multiple comparisons of salivary glucose for different groups of fasting plasma glucose (FPG) in patients

Group 1 FPG	Group 2 FPG	Significance
Less than 126 mg/dL	a. 126–180 mg/dL	0.007 [#]
	b. Greater than 180 mg/dL	<0.001 [#]
126–180 mg/dL	a. Less than 126 mg/dL	0.007 [#]
	b. Greater than 180 mg/dL	<0.001 [#]
Greater than 180 mg/dL	a. Less than 126 mg/dL	<0.001 [#]
	b. 126–180 mg/dL	<0.001 [#]

[#]The mean difference is significant at the 0.05 confidence level.

Table 4

Average levels of salivary glucose for different levels of HbA1c in patients

HbA1c levels	N	Salivary glucose (mg/dL)		f value
		Mean	Standard deviation	
Upto 7%	53	1.82	1.38	208.87 p<0.01 ^{##}
7%–8%	16	3.6	2.41	
Greater than 8%	31	9.07	1.33	
Total	100			

^{##} p value statistically significant.

Table 5

Multiple comparison of salivary glucose for different groups of HbA1c in patients

Group 1 HbA1c	Group 2 HbA1c	Significance (P value)
Upto 7%	a. 7%–8%	<0.01 ⁺
	b. Greater than 8%	<0.01 ⁺
7%–8%	a. up to 7%	<0.01 ⁺
	b. Greater than 8%	<0.01 ⁺
Greater than 8%	a. up to 7%	<0.01 ⁺
	b. 7%–8%	<0.01 ^{+,^}

⁺ The mean difference is significant at the 0.05 level.

The correlation of salivary glucose with FPG and HbA1c was studied in all the participants of the study. The correlation was found to be strong and significant as shown in Table 6. Table 7 shows that, when the correlation was studied separately for patients and controls, it was still

found to be strong and statistically significant. However, the correlation of salivary glucose with HbA1c in controls was slightly weaker when compared to other correlations but was still statistically significant. This correlation is represented in Figure 1 and Figure 2.

Table 6
Correlation of salivary glucose with FPG and HbA1c in the participants of the study

Parameter		FPG	HbA1c
Salivary glucose	Pearson Correlation	0.88	0.91
	Significance (2 tailed)	<0.01 *	<0.01*

* Correlation is significant at the 0.01 level (2-tailed).

Table 7
Correlation of salivary glucose with FPG and HbA1c in patients and controls

Group	Parameter		FPG	HbA1c
a. Patients	Salivary glucose	Pearson Correlation	0.9	0.89
		Significance (2 tailed)	<0.01 **	<0.01**
b. Controls	Salivary glucose	Pearson Correlation	0.9	0.66
		Significance (2 tailed)	<0.01 **	<0.01**

** Correlation is significant at the 0.01 level (2-tailed).

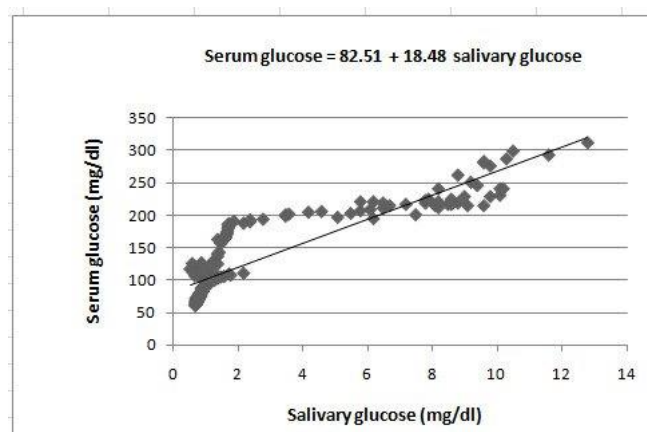


Fig. 1. Correlation between fasting salivary and serum glucose levels in the diabetic group.

Note: Regression coefficient was calculated and a formula was derived for the prediction of serum glucose from salivary glucose in the diabetic group.

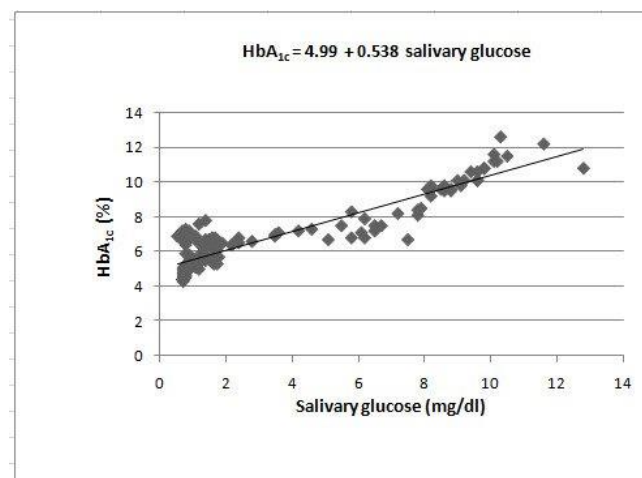


Fig. 2. Correlation between fasting salivary glucose levels and glycated hemoglobin (HbA1c) in the diabetic group.

Note: Regression coefficient was calculated, and a formula was derived for the prediction of HbA1c from salivary glucose in the diabetic group.

The regression coefficient was calculated and reflects the change in the FPG and HbA1C when there is a single unit change in salivary glucose. A formula (Figure 1 and Figure 2) was derived for prediction of FPG and HbA1c by salivary glucose using the regression equations:

- Serum glucose (in mg/dL) =
 $82.41 + 18.48 * \text{Salivary glucose (in mg/dL)}$
- HbA1c (in %) = $4.99 + 0.538 * \text{Salivary glucose (in mg/dL)}$

DISCUSSION

Diabetes mellitus is a chronic condition which affects the intermediary metabolism. The complications associated with diabetes mellitus are long term and affect various organ systems of the body. India being the diabetic capital of the world; diabetes mellitus is a major burden on the health services of the country.[10]

Currently, the WHO and American Diabetic Association recommend the evaluation of fasting blood glucose and HbA1C as the gold standard for the diagnosis and evaluation of diabetes mellitus. These parameters require invasive methods for blood collection which, when carried out at regular intervals of time, can be traumatic to the patients. Persons with diabetes require regular monitoring of blood glucose levels, the thought of which can cause anxiety to the patients.[11]

Further, the anxiety and trauma associated with venipuncture discourages the patients against regular evaluation of blood glucose levels. Keeping these factors in mind, a simple, non-invasive and inexpensive method for screening and prognosis evaluation of diabetes mellitus is the need of the hour.

Saliva is an ultra-filtrate of blood and is one the most copious secretions of the body. Also, its collection is easy and non-invasive. Keeping these factors in mind, saliva has a great potential to be used as a screening tool for various conditions.[12]

In the present study, we evaluated glucose levels in plasma and saliva. The evaluation was done in both individuals with diabetes and age matched controls. Glycated hemoglobin was also analyzed to assess the long-term glycemic status of the participants of the study.

The results of the study show that there is a significant difference between the salivary glucose levels in controls and individuals with diabetes. This indicates that a deranged glycemic status is reflected in the glucose levels of saliva. Also, the salivary

glucose increases in proportion with the increase in the FPG and HbA1C of the diabetic individual as shown in Tables 3 and 4. A strong and significant positive correlation of salivary glucose was seen with the FPG and HbA1c. Similar results were also obtained by Panda Abhikshyeet *et al.* They studied the correlation of serum glucose and salivary glucose in controls and individuals with diabetes. In the individuals with diabetes, the correlation of glycated hemoglobin and salivary glucose was also analyzed. They found that both correlations were highly significant and significant in individuals with diabetes and controls respectively.[13] Also, Mitsumori *et al.* manufactured a saliva analyzing system and concluded that salivary glucose can be used as a measure to estimate blood glucose level.[14]

The idea of measuring salivary glucose in the individuals with diabetes has been documented by other researchers as well. [15–17] However, some studies have reported that an increase in blood glucose has no influence on salivary glucose levels. Englander *et al.* concluded that it is unadvisable to replace plasma levels of glucose with parotid secretion for diagnosis of diabetes mellitus as the glucose concentration may be below the measurable range. [18] Lopez ME *et al.* did not find a significant correlation between salivary glucose and HbA1c levels in the study conducted by them.[19]

A similar strong and significant correlation between FPG and salivary glucose was also documented in the previous study by Choudhry *et al.* However, the sample size was smaller in that study [20] Also, Jha *et al.* deduced that the mean salivary glucose levels were higher in unstimulated saliva when compared to stimulated saliva of participants with controlled and uncontrolled diabetes [21].

The systematic review by Mascarenhas *et al.* reported an increase in salivary glucose concentrations with a stronger correlation for higher glycemic levels. This study stated that the poorer the glycemic control, the higher the salivary glucose levels. To put it briefly, some studies supported the idea of higher salivary glucose levels in diabetes whereas others opposed the same.[22]

Various theories have been proposed to explain the high glucose levels in the saliva of individuals with diabetes. Powers AC *et al.* explained the role of advanced glycation end products (AGEs) in the alteration of basement membrane leading to endothelial dysfunction. This alteration is responsible for the leakage of glucose in

the saliva secreted by the salivary glands.[23] However, researchers have suggested that a single phenomenon may not be able to explain the high glucose levels in saliva when the blood glucose levels are high for a long period of time. Apart from alteration in the permeability of the basement membrane, other factors like the leakage of glucose molecules via the gingival crevices and the small size of the glucose molecule may also play a role in the same.[24]

The results of our study conclude that there is a strong positive correlation between the plasma and salivary levels of glucose. Also, we can predict the glycemic status of an individual from the generated mathematical formula involving salivary glucose and FPG/HbA1c.

Screening programs can therefore be conducted wherein the salivary glucose levels could be analyzed to screen for individuals for diabetes mellitus. This would eliminate the need for invasive testing for identification of diabetes in a target population. Also, individuals with diabetes need regular invasive testing for intermittent analysis of blood glucose levels. This can be replaced with analysis of salivary glucose levels at regular

intervals of time combined with less frequent blood glucose analysis.[25]

Limitations of the study

Some limitations like oral retention of food, oral bacterial, utilization of glucose, medication taken in the previous night, undiagnosed parotitis and periodontitis may influence the results and therefore have to be kept in mind during analysis.[26] Further research in the area with greater number of subjects, study of influence of insulin, oral hypoglycemic agents and other drugs on salivary glucose would help in establishing the practical utility of salivary glucose analysis.

CONCLUSION

Keeping these points in mind, saliva hence can be used as an initial screening tool for diabetes mellitus. Standardization of the techniques for the estimation of salivary glucose and setting up a reference range will also make it useful in diagnosing diabetes mellitus.

Introducere: Prognosticul diabetului zaharat este dat de o mai bună monitorizare după diagnosticul bolii. Aceasta necesită venopuncție regulată ceea ce poate duce la anxietate din partea pacientului. Așadar, este necesară dezvoltarea unei metode de monitorizare mai puțin invazive, ce ar fi și eficientă financiar pentru sistemele de sănătate. Scopul studiului a fost de a măsura glucoza plasmatică și de a o corela cu glucoza salivară și HbA1c la pacienți diabetici și martori sănătoși.

Metode: Glicemia și glucoza salivară au fost determinate prin tehnica GOD-POD, HbA1c prin HPLC. Analiza statistică a fost evaluată prin SPSS.

Rezultate: S-a observat o diferență semnificativă statistic între glucoza salivară a pacienților cu diabet și a martorilor sănătoși. Totodată, nivelurile acestora se corelează pozitiv cu FPG și HbA1c. Folosind glucoza salivară, o ecuație de regresie a prezis FPG și HbA1c.

Concluzii: Saliva este utilă pentru monitorizarea diabetului zaharat. Standardizarea tehnicii și găsirea unei valori prag sunt necesare.

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Authors' contributions:

Dr Afreen Arshad Choudhry: Concept and design of study, acquisition, analysis and interpretation of data, manuscript preparation, manuscript editing and final approval before publishing.

Dr Preeti Sharma: Concept and design of study, interpretation of data, manuscript review, final approval of the version to be published.

Dr Mahendra Prasad: Concept and design of study, interpretation of data, manuscript review, final approval of the version to be published.

Dr Pradeep Kumar: Concept and design of study, interpretation of data, manuscript review, final approval of the version to be published.

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Ethics approval: Approval was obtained from the Institutional ethics committee of Santosh deemed to be university. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent for publication: The authors affirm that human research participants provided informed consent for publication.

REFERENCES

1. INTERNATIONAL DIABETES FEDERATION. *IDF Diabetic Atlas*, 9th Edition. Accessed 2019.
2. WORLD HEALTH ORGANIZATION. *Global report on diabetes*. Geneva: World Health Organization; 2016.
3. TRIPATHY JP, THAKUR JS, JEET G, CHAWLA S, JAIN S, PAL A, et al. *Prevalence and risk factors of diabetes in a large community-based study in North India: results from a STEPS survey in Punjab*, India. *Diabetol Metab Syndr*. 2017; **23**;9:8.
4. SMRITI K, PAI KM, RAVINDRANATH V, GADICHERLA S, PENTAPATI KC. *Salivary Glucose as a Diagnostic Marker for Diabetes Mellitus*. *J Diabetes Sci Technol*. 2016;**10**(4):991–992.
5. RAGUNATHAN H, ASWATH N, SARUMATHI T. *Salivary glucose estimation: A noninvasive method*. *Indian J Dent Sci* 2019;**11**:25–7.
6. CARVER CD. *Comparison of protein composition in stimulated vs. unstimulated whole human saliva*. Oregon Health and Science University; 2007.
7. MILLER CS, FOLEY JD, BAILEY AL, CAMPELL CL, HUMPHRIES RL, CHRISTODOULIDES N, et al. *Current developments in salivary diagnostics*. *Biomark Med* 2010;**4**(1):171e89.
8. AMBADE VN, SHARMA YV, SOMANI BL. *Methods for estimation of blood glucose: A comparative evaluation*. *Med J Armed Forces India*. 1998;**54**(2):131–133.
9. KARAMI A, BARADARAN A. *Comparative evaluation of three different methods for HbA(1c) measurement with High-performance liquid chromatography in diabetic patients*. *Adv Biomed Res*. 2014;**3**:94.
10. ANJANA RM, PRADEEPA R, DEEPA M, DATTA M, SUDHA V, UNNIKRISHNAN R, et al. *Prevalence of diabetes and prediabetes (impaired fasting glucose and/ or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study*. *Diabetologia*. 2011;**54**(12):3022–7.
11. AMERICAN DIABETES ASSOCIATION. *Diagnosis and classification of diabetes mellitus*. *Diabetes Care*. 2010;**33** Suppl 1(Suppl 1):S62–9.
12. DHANYA M, HEGDE S. *Salivary glucose as a diagnostic tool in Type II diabetes mellitus: A case-control study*. *Niger J Clin Pract*. 2016;**19**(4):486–90.
13. PANDA ABIKSHYEET, VENKATAPATHY RAMESH, NIRIMA OZA. *Glucose estimation in the salivary secretion of diabetes mellitus patients*. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. 2012;**5**:149–154.
14. MITSUMORI M, YAMAGUCHI M, KANO Y. *A new approach to noninvasive measurement of blood glucose using saliva analyzing system*. *Conf Proc IEEE Eng Med Biol Soc*. 1998;**4**:1767–1770.
15. AYDIN S. *A comparison of gherlin, glucose, alpha-amylase and protein levels in saliva from diabetics*. *J Biochem Mol Biol*. 2007;**40**(1):29–35.
16. LOPEZ ME, COLLOCA ME, PAEZ RG, SCHALLMACH JN, KOSS MA, CHERVONAGURA A. *Salivary characteristics of diabetic children*; *Braz Dent J*. 2003;**14**(1):26–31.
17. AMER S, YOUSUF M, SIDDIQUI PQ, ALAM J. *Salivary glucose concentrations in patients with diabetes mellitus – a minimally invasive technique for monitoring blood glucose levels*. *Pak J Pharm Sci*. 2001;**14**(1):33–37.
18. ENGLANDER HR, JEFFAY AI, FULLER JB, CHAUNCEY HH. *Glucose concentrations in blood plasma and parotid saliva of individuals with and without diabetes mellitus*. *J Dent Res*. 1963;**42**:1246.
19. LOPEZ ME, COLLOCA ME, PAEZ RG, SCHALLMACH JN, KOSS MA, CHERVONAGURA A. *Salivary characteristics of diabetic children*; *Braz Dent J*. 2003;**14**(1):26–31.
20. CHOUDHRY AA, S. G. RAJESHWARI, GURURAJA A, PRABHU K, SMRITI K, M PAI K. *Glucose and Antioxidants in the Saliva of Diabetic Patients*. *International Journal of Current Medical and Applied sciences*. 2015;**7**(1):50–53.
21. JHA S, DAVID C, SALUJA I, VENKATESH D, CHAUDHARY S. *Estimation of salivary glucose level and plasma glucose level in subjects with and without diabetes mellitus: a comparative study*. *Natl J Integrated Res Med*. 2013;**5**:65e70.
22. MASCARENHAS P, FATELA B, BARAHONA I. *Effect of Diabetes Mellitus Type 2 on Salivary Glucose – A Systematic Review and Meta-Analysis of Observational Studies*. *PLoS ONE*. 2014; **9**(7): e101706.
23. POWERS AC. *Diabetes mellitus*. In: Fauci A, Braunwald E, Kasper D, et al., editors. *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill; 2008:2275–2304.
24. LADGOTRA A. *Estimation of Salivary and Serum Biomarkers in Diabetic and Non Diabetic Patients - A Comparative Study*. *J Clin Diagnostic Res*. 2016;**10**:ZC56-ZC61.
25. TIONGCO RE, BITUIN A, ARCEO E, RIVERA N, SINGIAN E. *Salivary glucose as a non-invasive biomarker of type 2 diabetes mellitus*. *J Clin Exp Dent*. 2018;**10**(9):e902–7.
26. PANCHBHAI AS. *Correlation of salivary glucose level with blood glucose level in diabetes mellitus*. *J Oral Maxillofac Res*. 2012;**3**(3):e3.

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