DOI: 10.7860/JCDR/2015/11633.5971

Original Article

Dentistry Section

Estimation of Salivary Glucose and Glycogen Content in Exfoliated Buccal Mucosal Cells of Patients with Type II Diabetes Mellitus

RATHY RAVINDRAN[1], DEEPA MOOTHEDATHU GOPINATHAN[2], SUNIL SUKUMARAN[3]

ABSTRACT

Background: Diabetes mellitus is a common metabolic disorder which shows an increasing incidence worldwide. Constant monitoring of blood glucose in diabetic patient is required which involves painful invasive techniques. Saliva is gaining acceptance as diagnostic tool for various systemic diseases which can be collected noninvasively and by individuals with limited training.

Aim: The aim of the present study was to analyse the possibility of using salivary glucose and glycogen content of buccal mucosal cells as a diagnostic marker in Type II Diabetes mellitus patients which can be considered as adjuvant diagnostic tool to the gold standards.

Materials and Methods: Sample consists of 30 study and 30 control groups. Saliva was collected by passive drool method. Intravenous blood samples were collected for glucose estimation. Exfoliated buccal mucosal cells were collected from apparently normal buccal mucosa, smeared on dry glass slide and stained with PAS. Blood and salivary glucose are estimated by Glucose Oxidase endpoint method. For Glycogen estimation, number of PAS positive cells in fifty unfolded cells was analysed.

Results: The results of the present study revealed a significant increase in the salivary glucose level and the number of PAS positive buccal mucosal cells in the diabetics than in the controls. The correlation between the fasting serum glucose and fasting salivary glucose and also that between fasting serum glucose and PAS positive cells was statistically significant. But the correlation between the staining intensity and fasting serum glucose was statistically insignificant.

Conclusion: With the results of the present study it is revealed that salivary glucose and PAS positive cells are increased in diabetics which can be considered as adjuvant diagnostic tool for Diabetes mellitus.

Keywords: Diagnostic tool, PAS positive cells, Saliva

INTRODUCTION

The incidence of Diabetes Mellitus worldwide is increasing and will be one of the leading causes of mortality and morbidity for the foreseeable future. Wild S et al., estimated the global prevalence for year 2000 and reported that the prevalence of Diabetes mellitus for all age group worldwide was estimated to be 2.8% in 2000 and will be 4.4% [1].

Proper monitoring of blood glucose aids in early diagnosis and timely intervention to prevent diabetic complications. The monitoring of glycemic control in diabetic patient involves diagnostic procedures of analysis of constituents of blood which requires invasive techniques. The noninvasive glucose detection techniques offer several advantages because of fewer patient compliance problem and cost effective method for screening of large population. Saliva has been investigated reliably for reflecting and monitoring blood glucose concentration in diabetic patients by several investigators [2-5]. Hallikerith S et al., showed that PAS positive oral mucosal cells were significantly greater in diabetics than in the controls. Asemi-Rad A et al., showed that diabetes cause changes in glycogen of oral mucosal cells. They postulated that a decrease in Glycogen Synthase Kinase-3(GSK-3) enzyme phosphorylation lead to glycogen accumulation in cells [6,7]. Hence, the present study was undertaken to analyse the possibility of using salivary glucose and glycogen content of buccal mucosal cells as diagnostic aid in Type II Diabetes Mellitus patients.

MATERIALS AND METHODS

In this case control study, study group consisted of 30 diabetic patients and the control group consisted of 30 age matched healthy subjects. The members of the study group was selected from the patients attending the diabetic clinic of Azeezia Medical College, Meeyannoor, Kollam during the period of March 2013 to September 2013. Patients were selected by simple random sampling according to inclusion and exclusion criteria. The study was commenced upon obtaining clearance from institutional ethics committee. Prior to initiating the study, a written informed consent in accordance with ethical codes adopted by National Committee for Medical Research Ethics was completed by all participants. A detailed case history was taken from each study subject as per the proforma which consists of patient history regarding duration of the disease, type of glycemic control, family history and personal history.

Patients with known Type II Diabetes Mellitus for more than one year with FBS≥126mg/dl and with no other systemic illness other than Type II Diabetes Mellitus were included in the study. Patients with recently diagnosed diabetes with a duration of less than one year and with other systemic illness, under medication for other diseases other than Diabetes mellitus, Gestational and Type I Diabetes Mellitus, history of alcoholism, chewing and smoking tobacco habits and with a history of oral mucosal lesions were excluded from study. Procedure after confirmation of the disease by expert endocrinologist, 1.5ml of saliva and 1.5 ml of venous blood samples were collected 8 hours following fasting [8].

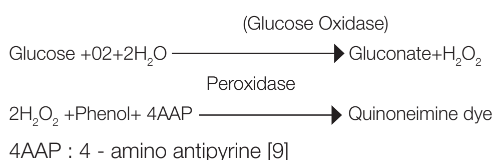
Under aseptic conditions using a sterile disposable 25 gauge needle, intravenous blood was collected from the median vein. Saliva collection was done using passive drool method. The unstimulated saliva was collected from patient between 8.00am and 10.00am and if the patient was a denture wearer, denture was removed prior to saliva collection. The patients were then asked to rinse their mouth

thoroughly with water 10 min prior to saliva collection and they were asked to spit out or swallow saliva already present in the mouth After the subjects were comfortably seated and after a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and drool all the saliva they produced into a vial using a custom made saliva collecting funnel over a period of 5-10 min till approximately 1.5 ml of saliva was collected. Once collected, saliva containing vials were placed in an ice carrier box and transferred to laboratory for biochemical analysis. Glucose estimation in both saliva and blood sample was done using the GOD-POD enzymatic method. Saliva sample was centrifuged at 3000 rpm for 15 min to remove any particulate material. The supernatant was taken for glucose estimation. For estimation of blood glucose, venous blood was allowed to clot in the test tube, centrifuged at 3000 rpm for 10 min and then serum was separated for glucose estimation. Glucose estimation was performed using glucose assay kit, GLUCOSE-LS (Euro Diagnostic Systems Pvt. Ltd.) SA kit. Briefly, 1ml of reagent solution was pipetted into test tube and 0.01ml saliva added. To another test tube add 1ml of standard and then 0.01ml of saliva. After mixing these tubes were incubated at 37° C for 10 min. The absorbance of these solutions was measured against the blank without dilution at a wavelength of 505 nm (Hg 546 nm)/Green within one hour of preparation. The glucose concentration was calculated as per the equation.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test (Abs.T)} \quad \text{X Glucose (mg/dl) in Standard}}{\text{Absorbance of standard (Abs.S)}}$$

Results were expressed as milligrams per deciliter (mg/dl).

Assay principle: Glucose is oxidized to gluconic acid and hydrogen peroxide by Glucose Oxidase. The hydrogen peroxide released is coupled with Phenol and 4- Aminoantipyrine (4-AAP) to form the coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm, the intensity of colour is directly proportional to glucose concentration in the sample [9].



4AAP : 4 - amino antipyrine [9]

Collection of exfoliated buccal mucosal cells for estimation of PAS positive cells: Patients were asked to rinse the oral cavity with water. Buccal mucosal cells were obtained by scraping the buccal mucosa with gentle pressure using a moistened wooden spatula from both study and control groups. The scrapings were smeared on the clean, fresh, dry glass slides and spread over a large area preventing clumping of cells. The slides were immediately sprayed with Biofix TM spray fixative to ensure proper fixation and stained with PAS. Fifty unfolded cells with a clear outline and predominant staining in each prepared smear were selected in a stepwise manner moving the microscope stage from left to right and then down and across in a zigzag manner in order to avoid counting the same cell again. The PAS-stained smears were analyzed for the presence of magenta coloured glycogen. Fifty cells were analyzed in each smear, and values were given as number of PAS-positive cells/50 cells. Exfoliated cells were also coded for staining intensity.

Coding of staining intensity:-

Code 1-Mild staining
Code 2-Moderate staining
Code 3-Intense staining

STATISTICAL ANALYSIS

Data analysis was performed using the software, Statistical Package for Social Sciences (SPSS Version 21). Unpaired student t test was used to assess the statistical significance among different parameters of the test and control group. Pearsons correlation coefficient test was applied to assess the correlation between Fasting Serum Glucose and Fasting Salivary Glucose, PAS positive cells and Fasting Serum Glucose, Staining intensity and Fasting Serum Glucose. Non parametric Mann Whitney U test was used for comparison of intensity of staining between the two groups. The data were expressed as mean (Standard Deviation{SD}) and in the entire tests p-value of ≤0.05 was taken to be statistically significant. ROC curves were plotted for assessing the effectiveness of salivary glucose level and PAS positive cells in the diagnosis of Diabetes Mellitus.

RESULTS

The sample of this study consisted of 30 diabetic patients and 30 healthy controls. Fasting serum glucose level ranged between 126 to 530 mg% in diabetic patients and 70-99mg % in control subjects. The mean level of fasting serum glucose in diabetic patients and controls are given in [Table/Fig-1]. The mean level of fasting salivary glucose in diabetic patients and healthy controls are given in [Table/Fig-2].

The comparison of fasting salivary glucose between diabetic group and control group demonstrated a highly significant increase in salivary glucose levels in diabetic group with a p-value of 0.001 [Table/Fig-3].The Correlation of fasting salivary glucose with fasting serum glucose in study group demonstrated a positive significant correlation with a p-value of 0.031 [Table/Fig-4].

The buccal mucosal cells were analysed for PAS positivity and the mean value of total number of PAS positive cells in diabetic group and in the controls are given in [Table/Fig-5]. The comparison of PAS positive cells between diabetic group and control group demonstrated a highly significant increase of PAS positive cells in diabetic group with a p-value of 0.001 [Table/Fig-6]. Correlation of PAS positive cells with fasting serum glucose in diabetic group demonstrated a positive significant correlation with p-value 0.043 [Table/Fig-7].

Parameter	Group	Mean	SD
Serum Glucose (mg/dl)	Diabetics	230.067	82.9794
	Controls	92.500	7.0991

[Table/Fig-1]: Distribution of mean levels of fasting serum glucose in diabetic group and healthy control group

Parameter	Group	Mean	SD
Salivary Glucose (mg/dl)	Diabetics	6.567	3.0477
	Controls	1.867	0.9732

[Table/Fig-2]: Distribution of mean levels of fasting salivary glucose in diabetic group and control group

Parameter	t-value	Mean difference	Standard Error difference	p- value
Salivary glucose	8.046	4.7000	0.5841	0.001

[Table/Fig-3]: Comparison of fasting salivary glucose in diabetics and controls
(S)-Significant

Parameter	Pearson correlation	p-value
Fasting Salivary Glucose/ Fasting Serum Glucose	0.394	0.031

[Table/Fig-4]: Correlation of fasting salivary glucose with fasting serum glucose in study group
Correlation is significant at 0.05 level

Parameter	Group	Mean	SD
Number of PAS positive cells	Diabetics	2.467	1.8333
	Controls	0.933	1.3113

[Table/Fig-5]: Distribution of mean value of PAS positive cells in diabetic group and healthy control group

Parameter	t-value	Mean difference	Standard Error difference	p- value
Number of PAS positive cells	3.726	1.5333	0.4115	0.001

[Table/Fig-6]: Comparison of PAS positive cells in diabetics and controls
(S)-Significant

Parameter	Pearson correlation	p-value
PAS positive cell/ Fasting Serum Glucose	0.372	0.043

[Table/Fig-7]: Correlation of total number of PAS positive cells with fasting serum glucose in study group

Parameter	Control	Diabetics	Mann- Whitney U	p-value
PAS intensity	0.93±1.311	2.467±1.833	223.500	0.001

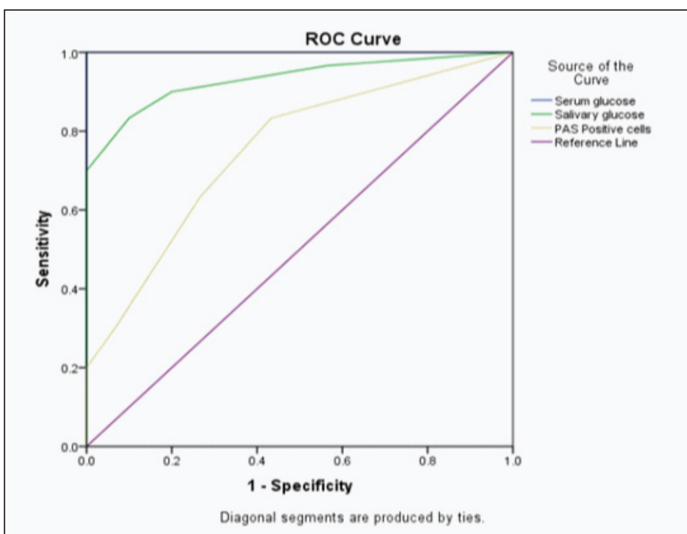
[Table/Fig-8]: Comparison of intensity of PAS staining in diabetics and controls

Parameter	Pearson correlation	p-value
Staining intensity/ Fasting Serum Glucose	0.259	0.168

[Table/Fig-9]: Correlation of staining intensity with Fasting Serum Glucose in study group
(NS)- Not Significant

Area Under Curve (AUC)			
Parameter	Area	Standard Error	p-value
Salivary Glucose	0.932	0.034	0.001
PAS positive cell	0.752	0.063	0.001

[Table/Fig-10a]: Area Under Curve



[Table/Fig-10b]: ROC Curve showing the predictive value of salivary glucose and PAS positive cells in the diagnosis of Diabetes Mellitus

Comparison of PAS staining intensity between diabetic group and control group demonstrated significant increase in intensity in diabetics when compared to control with a p-value of 0.001 [Table/Fig-8]. Correlation of PAS staining intensity with fasting serum glucose in study group demonstrated an insignificant relation between staining intensity and Fasting serum glucose [Table/Fig-9].

An ROC curve was drawn to demonstrate the predictive value of salivary glucose and PAS positive cells in the diagnosis of Diabetes Mellitus in [Table/Fig-10a,b] with AUC. Since the area under the curves is near to 1, salivary glucose & PAS Positive cells can be used as a diagnostic tool in Diabetic Mellitus.

DISCUSSION

Diabetes mellitus is a common metabolic disorder which requires routine monitoring of blood glucose for effective management and prevention of complications. The blood glucose monitoring requires invasive diagnostic techniques. Saliva, which can be collected by noninvasive techniques, is gaining interest as diagnostic tool for various systemic diseases because of fewer patient compliance problem and cost effective method of screening large population [10].

On analysis of saliva sample from study and control group it was found that the salivary glucose levels in diabetic patients were higher as compared to salivary glucose levels in controls in their fasting state. The results were comparable to the observations made by Al-Zahawi S et al., which showed a significant increase in salivary glucose in diabetics than in control [11]. In the study by Panchbai A S et al., the mean salivary glucose level in control was 1.89 which is similar to our value of 1.867 [12]; In the study group the mean value for salivary glucose was 6.56, which is also similar to their result in controlled diabetics with a value of 7.64, but in uncontrolled diabetics value was a slightly higher 8.09.

In contrary to the present study Gheena S et al., obtained a higher salivary glucose value in nondiabetic children than in diabetic children [13]. There was no significant correlation between salivary glucose and blood glucose within diabetic group but within nondiabetic group. Studies by Jurysta C et al., [14], Soares MS et al., [15], Nagalaxmi V et al., [16] Al- Zahawi SM et al., [11], Darwazeh et al., [17], Agha-hosseini F et al., [18], disproved any significant difference in salivary glucose levels between males and females. But there were also some studies which showed a gender difference in salivary glucose levels [12]. It is also worth mentioning that in diabetic group there was a corresponding increase in fasting salivary glucose levels as compared to fasting serum glucose levels and this was statistically significant (p=0.031) as well.

Most of the studies confirmed the association of increased glucose concentration in saliva and diabetes mellitus patients utilising either passive drool or spitting technique for saliva collection [19]. The present study adopted passive drool method. In the present study a significant increase in salivary glucose in diabetic patients when compared to healthy controls was noted. It was also noted that there was a significant correlation of salivary glucose level with serum glucose level. But in the study by Forbat et al., [2], the results showed that salivary glucose concentration was independent of blood glucose concentration. This was in keeping with the findings of Campbell [20], Von Mahr et al., [21] and Mehrotra & Chawla [22].

Method of saliva collection also varied between different investigators. While Forbat et al., collected specific parotid saliva; other investigators like Campell, Von Mahr et al., and Mehrotra & Chawla relied on whole mixed saliva samples [19]. In the present study unstimulated whole saliva samples was collected because of the ease of collection and also higher patient compliance. The stimulated saliva sample may give an inaccurate measurement of saliva constituents because of increased dilution. But in the study by Jurysta C et al., an increased glucose concentration in both stimulated and unstimulated saliva of diabetics when compared to non diabetics was obtained [14].

Many of these recent studies were conducted with the hope of finding out a non invasive method for glucose monitoring in diabetics as well as to answer the questions like whether we can conveniently use saliva sample as a means of diagnosis. A comparative analysis between saliva and blood in the same individual can help us discover the importance of saliva as a diagnostic test [23]. The advantage of using salivary assays over blood assays are, the sampling is very easy to do especially in non medical environment; multiple samples could be collected providing more information than that of single blood sample

A Positive correlation between salivary and serum glucose supports its use as a diagnostic tool. Some investigators like, Amer S et al., [24], Hegde A et al., [3], Naik Veena V et al., [25], Mahdavi SO et al.,[26] Abikshyeet P et al., [4], Panchbai AS et al., [27], Mirzaii-Dizgah I et al., [28] were able to demonstrate a positive correlation between salivary and serum glucose. Unlike their results, studies by Akanji AO et al., [29], Karjalainen KM et al., [30], Soares MS et al., [15] could not find any correlation between these two entities.

In the present study whole saliva was centrifuged and supernatant was taken for glucose estimation. Even though investigators like Campbell used similar methods, only 56.8% of normal controls and

66.7% of diabetics had measurable glucose where as glucose was definitely detectable in all our samples involving controls as well as diabetics even in minute amounts [2].

GOD-POD enzymatic method for glucose estimation was adopted in the present study which was similar to the study by Sathya priya S et al., [31]. Twetman et al., [32] also used the enzymatic method of glucose estimation but with hexokinase and glucose 6 phosphate dehydrogenase.

Carda C et al., estimated various salivary biochemical parameters along with salivary glucose and observed that longer duration of the disease leads to fatty infiltration and micro angiopathies of salivary glands [33]. In the present study patient with diagnosed diabetes for atleast one year were included. Carda C and co-workers observed an increase in salivary glucose levels in poorly controlled diabetics with plasma glucose ≥ 180 mg/dl and HbA1C ≥ 8%. The studies conducted by Twetman et al., [32] in young type 1 diabetics had also shown that an increase in HbA1C ≥ 8% coexisted with higher glucose levels in saliva. Reuterving CO et al., observed a positive correlation between parotid saliva and blood glucose levels in type 1 diabetics followed by a decrease in salivary glucose concentration during their period of improved metabolic control [5]. Raised glucose level was also recorded in gingival fluid of diabetics, thus proving that glucose in saliva may not be exclusively of salivary gland origin [34].

Jurysta C et al., studied the dependency of salivary glucose concentrations on glycemia during oral glucose tolerance test (OGTT) in both diabetic and non-diabetic subjects. A two fold increase in salivary glucose was observed within first 60 min followed by decrease in salivary glucose concentrations during successive sampling despite unaltered salivary flow [14].

The variability in the results of different workers may be a reflection of different population samples, different sampling techniques for collecting saliva or various methods for glucose analysis. Also the correlation between salivary glucose level and blood glucose level cannot be justified until salivary glucose estimation is authenticated. There should be homogeny in the methodology used. Also, the study sample needs to be standardised to have the study outcomes which will be comparable and sustainable

The present study also analysed the use of Glycogen positive cells in the exfoliated buccal mucosal cells as a screening marker for diabetes. Previously only two studies were carried out in this context and the present study is the first of its kind when done in conjunction with salivary glucose estimation. Smears were taken from buccal mucosa and stained with PAS for the demonstration of glycogen positive cells. The result showed a significant increase in the number of glycogen positive cells in diabetic group when compared to controls (p=0.001) which was similar to the study results by Hallikerimath S et al., [6] and Asemi –Rad A et al., [7]. A positive correlation of PAS positive cell and FBS levels were also noted in the present study with a p-value of 0.043 contradictory to the finding of Hallikerimath S et al.,[6].

We have also examined the intensity of PAS positivity along with number of PAS positive cells and it showed a significant increase in diabetics than controls. This is similar to study by Asemi –rad A et al., [7]. The current study demonstrated higher number of moderately stained cells than mild or intensely stained cells both in diabetic and control group. The correlation was looked upon between Fasting serum glucose and intensity of staining, and found an insignificant correlation (p=0.168).

The changes in morphometry of buccal exfoliated cells are already well established in diabetics. But examination of PAS positivity is comparatively an easy method without any sophisticated devices unlike performing cytomorphometry which is a tedious process. It seems that diabetes affects oral mucosa and it will be reflected in the staining property of cells. Thus it can be used as a complimentary or adjunct technique in the diagnosis of diabetes mellitus.

Although many studies have been carried out on salivary glucose estimation, the present study is the first of its kind combining two non-invasive procedures that can be easily and effectively performed for monitoring blood glucose level. The ROC curve was done to assess the predictability of these parameters in the diagnosis of Diabetes Mellitus. From ROC curve it was seen that Area under Curves are near to 1, so salivary glucose & PAS Positive buccal mucosal cells can be used as a diagnostic tool in Diabetic Mellitus.

CONCLUSION

This study reveals that salivary glucose and PAS positive cells are increased in diabetics which can be considered as adjuvant diagnostic tool for Diabetes Mellitus.

REFERENCES

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27(5):1047-53.

[2] Forbat LN, Collins RE, Maskell GK, Sonksen PH. Glucose concentrations in parotid fluid and venous blood of patients attending a diabetic clinic. *J R Soc Med*. 1981;74(10):725-28.

[3] Hegde A, Shenoy R, DMello P, Smitha A, Tintu A, Manjrekar P. Alternative markers of glycemic status in diabetes mellitus. *Biomedical Research*. 2010;21(3):252-56.

[4] Abikshyeet P, Ramesh V, Oza N. Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes Metab Syndr Obes*. 2012;5:149-54.

[5] Reuterving CO, Reuterving G, Hagg E, Ericson T. Salivary flow rate and salivary glucose concentration in patients with diabetes mellitus influence of severity of diabetes. *Diabete and Metabolisme*. 1987;13(4):457–62.

[6] Hallikerimath S, Sapra G, Kale A, Malur PR. Cytomorphometric analysis and assessment of periodic acid Schiff positivity of exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients. *Acta Cytol*. 2011;55(2):197-202.

[7] Asemi-Rad A, Heidari Z, Mahmoudzadeh-Sagheb H. The relationship between the staining intensity of oral exfoliative cells with periodic acid schiff and cytomorphometric indices with fasting blood sugar in type 2 Diabetic patients. *Interdisciplinary J Contemp Research Business*. 2013;4(9):375-81.

[8] American Diabetes Association. Diagnosis and Classification of Diabetes. *Diabetes Care*. 2009;32:S62-67.

[9] Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann Clin Biochem*. 1969;6:24-27.

[10] Kallapur BR, amalingam K, Bastian Mujib A, Sarkar A, Sethuraman S. Quantitative estimation of sodium, potassium and total protein in saliva of diabetic smokers and non-smokers: A novel study. *J Nat Sci Biol Med*. 2013;4(2):341-45.

[11] Al-Zahawi SM, Al-Barzenji HAM, Al-Qassab ZA. Effects of diabetes mellitus types II on salivary flow rate and some salivary parameters (total protein, glucose, and amylase) in Erbil city. *J Bagh Coll Dentistry*. 2012;24(sp 2):123-27.

[12] Panchbai AS, Degwekar S, Bhowte R. 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.

[13] Gheena S, Chandrasekhar T, Ramani P. Salivary characteristics of diabetic children. *Brazilian J Oral Sci*. 2011;10(2):93-97.

[14] Jurysta C, Bulur N, Oguzhan B, Satman I, Yilmaz TM, Malaisse WJ, et al. Salivary glucose concentration and excretion in normal and diabetic subjects. *J Biomed Biotechnol*. 2009; 2009:Article ID:430426.

[15] Soares MS, Batista-Filho MM, Pimentel MJ, Passos IA, Kustner EC. Determination of salivary glucose in healthy adults. *Med Oral Patol Oral Cir Bucal*. 2009;14:e510-13.

[16] Nagalaxmi V, Priyanka V. Can Saliva be a marker for predicting Type I Diabetes Mellitus?-A Pilot Study. *J Indian Acad Oral Med and Radiol*. 2011;23(4):579-82.

[17] Darwazeh AM, MacFarlane TW, McCuish A, Lamey PJ. Mixed salivary glucose levels and candidal carriage in patients with diabetes mellitus. *J Oral Pathol Med*. 1991;20(6):280-83.

[18] Agha-Hosseini F, Dizgah IM, Amirkhani S. The composition of unstimulated whole saliva of healthy dental students. *J Contemp Dent Pract*. 2006;7(2):104-11.

[19] Granger DA, Kivlighan KT, Fortunato C, Harmon AG, Hibel LC, Schwartz EB, et al. Integration of salivary biomarkers into developmental and behaviorally-oriented research: problems and solutions for collecting specimens. *Physiol Behav*. 2007;92(4):583-90.

[20] MJA Campbell. The glucosein the saliva of the non diabetic and the diabetic patient. *Arch Oral Biol*. 1965;10(2):197-205.

[21] Mohr G, Neumann P, Georgi K. The glucose content of saliva and sweat in normal and diabetic subjects. *Med Klin*. 1968;63(6):209-11.

[22] Mehrotra KK, Chawla TN. Quantitative estimation of salivary glucose. *J Indian Dent Assoc*. 1968;40(9):243-48.

[23] Lawrence HP. Salivary markers of systemic disease:non invasive diagnosis of disease and monitoring of general health. *J Can Dent Assoc*. 2002;68(3):1-74.

[24] Amer S, Yousuf M, Siddiqiui PQ, Alam J. Salivary glucose concentration in patients with diabetes mellitus- a minimally invasive technique for monitoring blood glucose levels. *Pak J Pharm Sci*. 2001;14(1):33-37.

[25] Naik VV, Satpathy Y, Pilli GS, Mithilesh NM. Comparison and correlation of glucose levels in serum and saliva of patients with diabetes mellitus. *Indian J Public Health Research & Development*. 2011;2:103-05.

[26] Mahdavi SO, Hashemi S, Boostani NS, Zokaee H. A new method to evaluate fasting plasma glucose by salivary glucose measurement. *Iranian J Diabetes and Obesity*. 2012;4(3):127-33.
[27] Panchbhai AS. Correlation of salivary glucose level with blood glucose level in Diabetes Mellitus. *J Oral Maxillofac Res*. 2012;3(3):e3.
[28] Mirzaii- Dizgah I, Mirzaii- Dizgah MR, Mirzaii- Dizgah MH. Stimulated saliva glucose as a diagnostic specimen for detection of diabetes. *J Archives Military Med*. 2013;1(1):24-27.
[29] Akanji AO, Ezenwaka C, Adejuwon CA, Osotimehin BO. Plasma and salivary concentrations of glucose and cortisol during insulin-induced hypoglycaemic stress in healthy Nigerians. *Afr J Med Med Sci*. 1990;19(4):265-69.
[30] Karjalainen KM, Knuuttila MLE, Kaar ML. Salivary factors in children and adolescents with insulin- dependent diabetes mellitus. *Paediatr Dent*. 1996;18(4):306-11.
[31] Sathya priya S, Bharani GO, Nagalingam M, Jayanthi M, Kanagavalli U. Potential of salivary protein as a biomarker in prognosis of Diabetes Mellitus. *J Pharmacy Research*. 2011;4(7):2228-29.
[32] Twetman S, Nederfors T, Stahl B, Aronson S. Two-year longitudinal observations of salivary status and dental caries in children with insulin-dependent diabetes mellitus. *Pediatr Dent.* 1992;14(3):184-88.
[33] Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. *Med Oral Patol Oral Cir Bucal*. 2006;11(4):E309-14.
[34] Yamaguchi M, Takada R, Kambe S, Hatakeyama T, Naitoh K, Yamazaki K, et al. Evaluation of time-course changes in gingival crevicular fluid glucose levels in diabetics. *Biomed Microdevices*. 2005;7(1):53-58.

PARTICULARS OF CONTRIBUTORS:
1. Professor and HOD, Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Science & Research, Meeyannoor, Kerala, India.
2. Post Graduate Student, Department of Oral & Maxillofacial Pathology, Azeezia College of Dental Science & Research, Meeyannoor, Kerala, India.
3. Professor, Department of Oral & Maxillofacial Pathology, Azeezia College of Dental Science & Research, Meeyannoor, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rathy Ravindran,
Professor and HOD, Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Science & Research,
Meeyannoor, Kerala, India.
E-mail : rathyravindran27@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Oct 22, 2014**
Date of Peer Review: **Mar 20, 2015**
Date of Acceptance: **Mar 29, 2015**
Date of Publishing: **May 01, 2015**