

Original Article

The use of salivary glucose concentration as an indicator for glycemic control in diabetic patients in Benghazi

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ABSTRACT

Objectives: The aim of this study was to evaluate saliva glucose in diabetics and healthy people and assess the possibility of using salivary glucose concentration (SGC) for monitoring glycemic control instead of glycated hemoglobin (Hb_{A1c}).

Subjects and Methods: The groups of study composed of: diabetic group consisted of 52 diabetic patients and 25 healthy subjects as a control group. After collection of saliva and blood samples, the SGC and fasting blood sugar (FBS) levels were measured with enzymatic-oxygen rate method by Beckman Glucose Analyzer II. Hb_{A1c} was measured by Cobas c 111- Roche.co. Independent samples t-test was used for the comparison of means. Pearson's r correlation coefficient was used to describe the magnitude and direction of the relationship. ANOVA (F test) and t-test were used to confirm the statistical significance and calculating the regression equation.

Results: The average of SGC in diabetic group was significantly higher than non-diabetic group, 17.14 ± 4.69 mg/dl and 14.08 ± 1.63 mg/dl, respectively, with ($p = 0.000$). There was no significant correlation in diabetic and non-diabetic groups between SGC and Hb_{A1c} ($p > 0.05$). We observed a significant correlation between FBS and Hb_{A1c} in diabetics ($p < 0.05$).

Conclusion: In this study, all salivary samples reflected presence of glucose in both groups. The average of SGC levels in diabetic group was significantly higher than non-diabetic subjects. In diabetics, there were no effects on the average of SGC with the change of gender, age, type of therapy and duration of the disease. SGC levels were not directly influenced by change in Hb_{A1c} levels.

Keywords: Diabetes Mellitus; Glycemic control; Saliva; glucose.

INTRODUCTION

Incidence of both microvascular and macrovascular complications have been increased with poorly controlled diabetes mellitus (DM). These include retinopathy with potential loss of vision and nephropathy with end stage renal failure, hypertension, hyperlipidemia, atherosclerotic cardiovascular disease, peripheral vascular disease and cerebrovascular disease, peripheral and autonomic neuropathies such as numbness and tingling of extremities, impaired wound healing and increased susceptibility to infections ⁽¹⁾.

Oral complications have been reported to be associated with DM. These include periodontal diseases, salivary dysfunction leading to a reduction in salivary flow, changes in saliva composition and taste dysfunction, oral fungal and bacterial infections, denture stomatitis, fissured tongue, oral lichen planus (OLP), lichenoid reaction and angular cheilitis ^(2, 3). In addition, delayed mucosal wound healing, mucosal neuro-sensory disorders, dental caries and tooth loss have been reported in patients with DM ⁽⁴⁾.

The prevalence and the chance of developing oral mucosal lesions were found to be higher in patients with DM compared to healthy controls ⁽⁵⁾.

Dentists have a role in the detection of undiagnosed diabetic patients throughout the presence of associated oral complications and they are responsible for management of the oral complications of the disease and promoting proper oral health behaviors that limit the risks of

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tooth loss, periodontal disease and soft-tissue pathologies ⁽⁶⁾.

Glycated hemoglobin (Hb_{A1c}) has become the best clinical measure of glycaemic control. Hb_{A1c} is the non-enzymatic glycation of adult haemoglobin. Hb_{A1c} is much higher in diabetic patients than in healthy people and patients with higher Hb_{A1c} levels are more risky for DM complications ⁽⁷⁾.

Several studies and researches have been done in order to evaluate the benefit of using saliva as a diagnostic tool for some diseases. Saliva is suitable for children and elderly, since whole saliva can be collected easily, non-invasively and no need for special equipments compared with the collection of blood. The present study was conducted to evaluate the benefit of saliva to measure glycemic control.

SUBJECTS AND METHODS

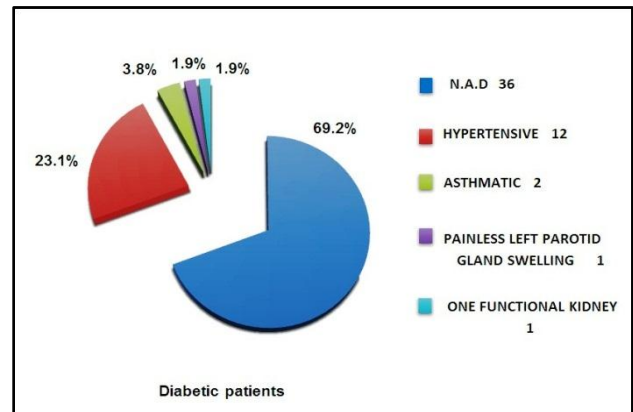
Study population

This observational case-control study was conducted at Benghazi Diabetic Centre. Ethical approval was taken from Faculty of Dental Surgery, university of Benghazi and from director of Benghazi Diabetic Centre. After explanation of nature of the study, written informed consent was taken from all participants. The groups of the study composed of two groups: diabetic group consisted of 52 diabetic patients that selected by stratified random sampling method. The sample design of diabetics consisted of two equal strata depending on gender (26 male and 26 female). Each stratum divided into two equal subgroups depending on type of therapy (insulin and oral hypoglycemic agents). Each final subgroup composed of 13 patients that were selected randomly up to the desired number. The ages of diabetics ranged from 23-75 years old. Patients were excluded only if they have impairment in motor and cognitive skills. Non-diabetic group selected randomly and consisted of 25 subjects with age ranged from 12-76 years old. It was composed of 16 female and 9 males (Figure 1).

Saliva and blood collection

All participants were given appointments for collection of samples at 8:30 a.m. participants must be fasting 8 hours before coming to the appointment. The brushing of the teeth was ordered from all participants the night before coming. Do not use dental brush, floss or mouth wash in the day of sample collection. The participant may rinse with water before saliva collection. On sitting in the dental chair, the subjects were asked to bend their

heads forward and downward and after an initial swallow, allow saliva flowing into the floor of the



NAD: Nothing Abnormality Detected.

Figure 1: Medical history of diabetic patients

mouth. Subjects will expectorate the saliva collected in the floor of the mouth into a test tube. During collecting the sample of saliva, the subject should be seated in a quiet room, not to cough or clear the throat into the collection tube. A sample of 1 cc of unstimulated whole saliva was collected. Blood samples were taken from each participant in the same visit. The collected samples were transferred to the lab for analyses after collecting immediately.

Glycosylated hemoglobin estimation

Hb_{A1c} level was determined by using of hemolyzing reagent (Tina-quant Hemoglobin A1c Gen.2 – Whole blood and Hemolysate Application) with (Cobas c 111) Roche.co device. Whole blood sample was taken without centrifugation for Hb_{A1c}.

Saliva and blood glucose estimation

The samples of saliva and blood were centrifuged in the lab at speed of 3000 rpm for 5 minutes in order to get clear saliva and serum. SGC and FBS levels were measured by using glucose oxidase enzyme and oxygen rate method via Beckman Glucose Analyzer II.

Statistical analysis

Statistic package for social science version 18 (SPSS) was used for analysis of data. All variables were assessed for normality of distribution. All variables were expressed as means $\pm$ standard deviations. Independent samples t-test was used for the comparison of means. Pearson's r correlation

coefficient and Regression line drawn on the scatter plot diagram were used to determine possible associations between variables. Results would be considered significant when $p < 0.05$. ANOVA (F test) and t-test were used to confirm the statistical significance and calculating the regression equation.

RESULTS

In terms of independent samples t-test, the mean SGC in diabetic group (17.14 ± 4.69 mg/dl) was significantly higher than non-diabetic group (14.08 ± 1.63 mg/dl), respectively, ($p = 0.000$) (Table 1).

Within diabetic group, no significant differences in mean SGC have been found between subgroups ($p > 0.05$) (Table 2). Insignificant correlation was found between SGC and Hb_{A1c} in diabetic and non-diabetic groups, ($r = -0.031$, $p = 0.825$), ($r = -0.054$, $p = 0.799$), respectively, (Table 3 and Figure 2 and 3). There was no significant correlation found between SGC and FBS in both groups. The only significant correlation was found in diabetic patients between FBS and Hb_{A1c} , ($r = 0.492$, $p = 0.000$) (Table 3 and Figure 4).

Table 1: comparison of Means of SGC in between diabetic and non-diabetic groups.

study groups		Hb_{A1c}	SGC	FBS
Diabetic	N	52	52	52
	Mean	8.7038	17.1346	142.7308
	Std. Deviation	1.66686	4.69054	46.58036
	Minimum	6.20	10.00	80.00
	Maximum	12.00	31.00	270.00
Non-diabetic	N	25	25	25
	Mean	5.7000	14.0800	80.8400
	Std. Deviation	.38079	1.63095	4.97226
	Minimum	5.00	11.00	73.00
	Maximum	6.40	17.00	91.00
P value		0.000**		

** $P = 0.000$ indicates highly significant.

Table2: comparison of mean of SGC between subgroups within diabetic group.

SGC	Diabetic subgroups	N	Mean	Std. Deviation	P value
Sex	Male	26	17.5000	4.74342	0.579
	female	26	16.7692	4.70155	
Type of therapy	Insulin	26	16.6154	4.31812	0.430
	Oral hypoglycemic	26	17.6538	5.06709	
Age	up to 60 years	36	17.7778	4.96336	0.140
	over 60 years	16	15.6875	3.75444	
Duration	up to 10 years	39	17.0513	4.66199	0.827
	over 10 years	13	17.3846	4.95880	

Table 3: The correlation coefficient between SGC, Hb_{A1c} and FBS.

study groups		SGC-Hb _{A1c}	SGC- FBS	Hb _{A1c} -FBS
Diabetic	r*	- 0.031	0.088	0.492
	P value	0.825	0.534	0.000 **
Non-diabetic	r*	- 0.054	- 0.327	- 0.033
	P value	0.799	0.110	0.876

* r indicates Pearson's r correlation coefficient.

** P=0.000 indicates highly significant.

DISCUSSION

The goal of achieving new techniques by using saliva to evaluate glycemic control in diabetic patients is saving time and creating a comfortable way instead of needle puncture to measure glycemic control which may lead to future development of such devices using saliva in dental and medical clinics.

In this study, a stratified random sampling method was used for selecting diabetic patients. This type of sample selection was mandatory to ensure representation of subgroups of interest or desired elements, including sex and each type of therapy. Since exact proportions of these elements in our study population were unknown, equal proportions of numbers selected to provide a desired balance of representation in the study⁽⁸⁾.

The present study revealed that the salivary samples in both diabetic and non-diabetic groups reflected presence of glucose in saliva with mean SGC (17.14 ± 4.69 mg/dl) and (14.08 ± 1.63 mg/dl), respectively, and this disagree with Amer et al.⁽⁹⁾ in which they reported the SGC level was only detected in diabetic patients. This study rely on the oxygen rate method (Sensitivity range ≥ 10 mg/dl) with the use of glucose oxidase (Glukar reagent) to measure glucose levels in both blood and saliva samples which was capable of glucose detection in lower concentrations even in non-diabetics. Mean SGC in non-diabetic group ranged from 11 mg/dl to 17 mg/dl which can be easily detected by using this method. Amer et al.⁽⁹⁾ have used a method that was not sensitive in case of lower glucose level (Sensitivity range > 20 mg/dl), but greater level of the glucose. Therefore, it is suitable for glucose detection in blood but not in saliva⁽¹⁰⁾. Oxygen rate method used in this study was more sensitive.

In this study, the average of SGC levels in diabetics was statistically significantly higher than

non-diabetic subjects ($p=0.000$) by using independent samples t-test (Table 1). It's also reported by many studies^(11-15, 10, 16, 17). Conversely, Vaziri et al.⁽¹⁸⁾ found no significant difference in mean SGC between diabetic and healthy control groups. This may be explained that glucose is small molecule could easily diffuse through semipermeable membranes and DM is often associated with increased basement membrane permeability which could be attributed to the increased passage of molecules from serum into exocrine glands secretions and through gingival crevicular fluids^(14, 19-21).

By dividing the diabetic group in this study into subgroups, the salivary glucose concentration (SGC) failed to differ significantly ($p>0.05$) in mean by using independent samples t-test between males and females ($p=0.579$), patients on insulin and oral hypoglycemic (0.430), patients aged less than or equal 60 years and over 60 years ($p=0.140$), and patients that have onset of diabetes since 10 years with those over 10 years ($p=0.827$). Therefore, according to this study, it can be postulated that there were no intra-group differences in mean SGC with change of gender, type of therapy, age of the patients and duration of the disease (Table 2).

In agreement with this study, Gupta et al.⁽²²⁾ and Soares et al.⁽²³⁾ did not find any significant difference of mean SGC with change of gender in diabetic group. Nevertheless, Darwazeh et al.⁽²⁴⁾ found higher levels of SGC in males as compared to females. Gupta et al.⁽²²⁾, Darwazeh et al.⁽²⁴⁾ and Sashikumar and Kannan⁽²⁵⁾ proved no significant effect on mean SGC with change of age in diabetic group. Regarding to the effect of duration of diabetes on SGC, the findings of this study revealed no significant difference in mean SGC that were similar to the findings reported by Gupta et al.⁽²²⁾ and Darwazeh et al.⁽²⁴⁾.

The study's results revealed that salivary glucose concentration (SGC) had no significant correlation with HbA_{1c} level by the use of Pearson's test, ($r=-0.031$, $p=0.825$) and ($r=-0.054$, $p=0.799$) for diabetic and control groups, respectively, that contrast with the aim of using SGC instead of HbA_{1c} (Table 3 and Figure 2 and 3).

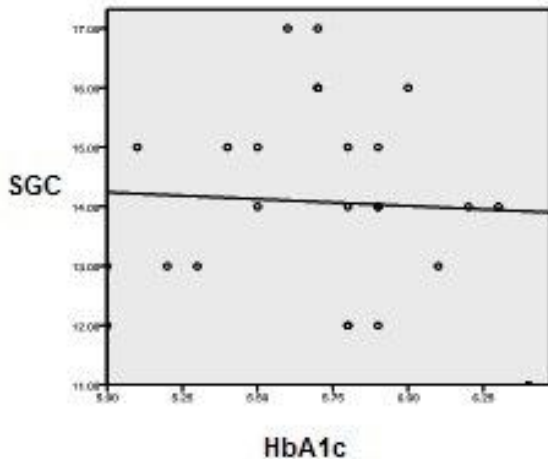


Figure 2: Regression line drawn on scatter diagram relating SGC with HbA_{1c} data in non-diabetic group.

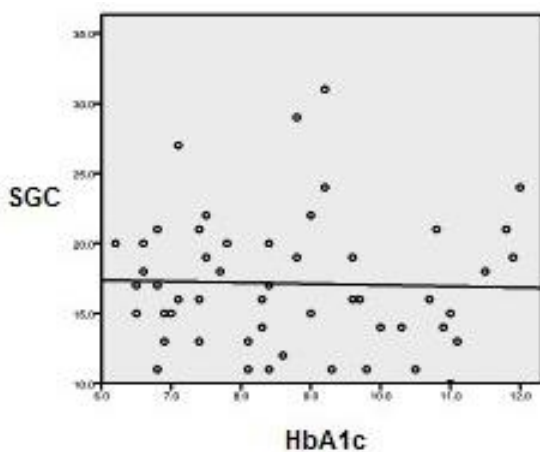


Figure 3: Regression line drawn on scatter diagram relating SGC with HbA_{1c} data in diabetic group.

Sashikumar and Kannan ⁽²⁵⁾ study also did not find a correlation between SGC and HbA_{1c} . The findings of this study were in contradiction with recent reports. Mahdavi et al. ⁽¹⁰⁾ reported a strong correlation between SGC and HbA_{1c} in diabetic group ($r=0.516$, $p=0.0001$) and no significant correlation in non-diabetic ($r=-0.112$, $p=0.454$).

Gupta et al. ⁽²²⁾ revealed that a significant correlation was found between SGC and HbA_{1c} levels in diabetic subjects ($P<0.001$). Abhikshyeet et al. ⁽²⁶⁾ also reported a significant correlation between SGC and HbA_{1c} levels. The criteria of exclusion of diabetics in the previous mentioned studies was based on excluding patients with any associated diseases, smoking, radiotherapy and other non-diabetic drugs which in turn may have a role in the discrepancies with the results of this study.

The levels of FBS were measured as an extra routine analysis for all participants. The present study have revealed that no significant relation found between FBS and SGC, ($r=0.088$, $p=0.534$) and ($r=-0.327$, $p=0.110$) for diabetic and control groups, respectively, (Table 3). These results were in agreement with some studies ^(13, 14, 18). On the other hand, other studies were contradicted with these results ^(12, 9, 15, 27).

A moderate positive correlation between FBS and HbA_{1c} in diabetic group was found in this study with ($r=0.492$, $p=0.000$) and ($R^2 = 0.242$). It was confirmed by using ANOVA F-test and t-test ($p<0.05$). From adjusted R^2 , (22.7%) of HbA_{1c} values could estimate or predict FBS values with a regression equation formula as:

$$(FBS) = 13.751 \times (HbA_{1c}) + 23.041$$

In contrast, there was no significant correlation between FBS and HbA_{1c} in non-diabetic control group ($r=-0.033$, $p=0.876$), and these results were supported by recent study of Khan et al. in 2015 ⁽²⁸⁾ (Table 3 and Figure 4).

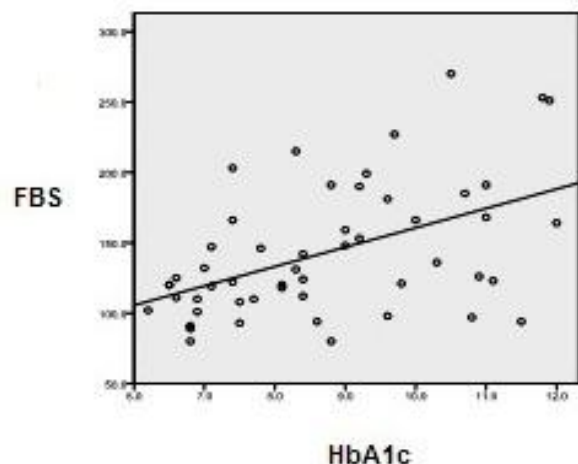


Figure 4: Regression line drawn on scatter diagram relating FBS with HbA_{1c} data in diabetic group.

CONCLUSION

In the light of the study results:

1. All salivary samples reflected presence of glucose in diabetic and non-diabetic groups.
2. The average of SGC level in diabetic group was significantly higher than non-diabetic subjects. In diabetics, there were no effects on the average of SGC with the change of gender, age, type of therapy and duration of the disease.
3. The present study could not support the use of saliva as an indicator for monitoring glycemic control because SGC levels were not directly influenced by change in Hb_{A1c} levels.

RECOMMENDATIONS

There are some limitations associated with the present study should be noted when analyzing the results:

1. The sample size of study groups is not very big that has been restricted by the cost of saliva and blood investigations; especially there were no sources of funding. Thus, availability of sources of funding would give a chance of increasing sample size with taking into account the use of another randomized method that would show representation of study population to good advantage.
2. Collecting of whole unstimulated saliva regardless of the degree of periodontitis may be associated with increase in glucose concentration from gingival crevicular fluids. More explorations regarding the effect of gingival crevicular fluids on salivary glucose concentration by determining the amount of effect would estimate more accurate levels of glucose molecules in saliva.

In future, more researches to be done using cohort study using large sample size of diabetic cases and taking into consideration that some of diabetics may receive antihypertensive drugs which may affect the salivary glands function. Effect of gingival crevicular fluids on salivary glucose concentration required to be more investigated.

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REFERENCES

1. Report of the expert committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetic care* 2000; 23:4-19.
2. Sandberg GE, Sundberg HE, Fjellstrom CA, Wikblad KF. Type 2 diabetes and oral health: A comparison between diabetic and non-diabetic subjects. *Diabetes Res Clin Pract* 2000; 50:27-34.
3. Guggenheimer J, Moore PA, Rossie K, Myers D, Mongelluzzo MB, Block HM, et al. Insulin dependent diabetes mellitus and oral soft tissue pathologies. II. Prevalence and characteristics of Candida and candidal lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; 89:570-6.
4. Lamster IB, Lalla E, Borgnakke WS, Taylor GW. The relationship between oral health and diabetes mellitus. *J Am Dent Assoc* 2008; 139:19-24.
5. Saini R, Al-Maweri SA, Saini D, Ismail NM, Ismail AR. Oral mucosal lesions in non-oral habit diabetic patients and association of diabetes mellitus with oral precancerous lesions. *Diabetes Res Clin Pract* 2010; 89:320-6.
6. Moore PA, Zgibor JC, Dasanayake AP. Diabetes: A growing epidemic of all ages. *J Am Dent Assoc* 2003; 134:11s-15s.
7. Day C, Bailey JC. HbA_{1c} - changing units. *Br J Diabetes Vasc Dis* 2009; 9:134-136.
8. Abounaja S. Lecture notes on biostatistics for medical and public health students. 2nd edition. National books store. Benghazi (2007).
9. Amer S., Yousuf M., Siddique PQR., Alam J. Salivary glucose concentrations in patients with diabetes mellitus-A minimally invasive technique for monitoring blood glucose levels. *Pakistan J Pharma Sci* 2001; 14(1):33-37.
10. Mahdavi SO, Hashemi S, Boostani NS, Zokaee H. A New Method to Evaluate Fasting Plasma Glucose by Salivary Glucose Measurement). *Iranian J of diabetes and obesity* 2012; 4(3):127-133.
11. Belazi, MA, Galli-Tsinopoulou A, Drakoulakos D, Fleva, A. Papanayiotou PJ. Salivary alterations in insulin dependent diabetes mellitus. *Int. J. Paediatr. Dent* 1998; 8: 29-33.
12. López ME, Colloca ME, Pérez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. *Braz Dent J* 2003; 14(1):26-31.
13. Jurysta C, Bulur N, Oguzhan B, Satman I, Yilmaz MT, Malaisse JW, Sener A. Salivary Glucose Concentration and Excretion in Normal and Diabetic Subjects. *J Biomed Biotech* 2009; Article ID 430426. 6 pages.
14. Vasconcelos ACU, Soares MSM, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. *J Oral Science* 2010; 52(2):293-298.



15. Panchbhair SA. Correlation of Salivary Glucose Level with Blood Glucose Level in Diabetes Mellitus. *J Oral Maxillofac Res* 2012; 3:1-7.
16. Lasisi TJ and Fasanmade AA. Salivary flow and composition in diabetic and non-diabetic subjects. *Niger. J. Physiol. Sci* 2012; 27: 079 – 082.
17. Aydin S. A Comparison of Ghrelin, Glucose, Alpha-amylase and Protein Levels in Saliva from Diabetics. *Journal of Biochemistry and Molecular Biology* 2007; 40(1): 29-35.
18. Vaziri PB, Vahedi M, Mortazavi H, Abdollahzadeh Sh, Hajilooi M. Evaluation of Salivary Glucose, IgA and Flow Rate in Diabetic Patients: A Case-Control Study. *Journal of Dentistry* 2010; 7(1).
19. Harrison R, Bowen WH. Flow rate and organic constituents of whole saliva in insulin-dependent diabetic children and adolescents. *Pediatr Dent*. 1987; 9(4):287-291.
20. Tenovuo J, Lehtonen OP, Vikari J, Larjava H, Vilja P, Tuohimaa P. Immunoglobulins and innate antimicrobial factors in whole saliva of patients with insulin-dependent diabetes mellitus. *J Dent Res*. 1986; 65(2):62-66.
21. Panchbhair 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):359-368.
22. Gupta S, Sandhu SV, Bansal H, Sharma D. Comparison of Salivary and Serum Glucose Levels in Diabetic Patients. *Journal of Diabetes Science and Technology* 2015; 9(1):91-96.
23. Soares MS, Batista-Filho MM, Pimentel MJ, Passos IA, Chimenos-Kustner E. Determination of salivary glucose in healthy adults. *Med Oral Patol Oral Cir Bucal*. 2009; 14(10):e510-e513.
24. 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):280-283.
25. Sashikumar R, Kannan R. Salivary glucose levels and oral candida carriage in type II diabetics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010; 109(5):706-711.
26. Abhikshyeet P, Ramesh V, Oza N. Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes Metab Syndr Obes*. 2012; 5:149-154.
27. Agrawal RP, Sharma N, Rathore MS, Gupta VB, Jain S, Agarwal V, Goyal S. Noninvasive Method for Glucose Level Estimation by Saliva. *J Diabetes Metab* 2013; 4(5):266.
28. Khan HA, Sobki SH, Alhomida AS. Regression analysis for testing association between fasting blood sugar and glycated hemoglobin in diabetic patients. *Biomedical Research* 2015; 26(3): 604-606.