A New Method to Evaluate Fasting Plasma Glucose by Salivary Glucose Measurement

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Received: 12 July 2012
Accepted: 10 September 2012

Abstract

Objective: Fasting plasma glucose (FPG) analysis is the most importantmethod for detection and diagnosis of diabetes mellitus. Due to difficulty and problems of this method for determination of glycemia in diabetic patients, recently the use of Fasting Salivary Glucoseas a simple and non-invasive method to evaluate FPGhas came into significant considerationof specialists. The aim of this study was the presentation of a new method to evaluate FPG by salivary glucose measurement

Material and Methods: This was a cross-sectional study which was done on 52 diabetic patients (test group) and 47 non diabetic patients (control group). After collection of saliva and blood samples, The FPG level was measured by GOD-PAP method and FSGlevel was measured by Glucose oxidase/peroxidase method. The statistical significance was calculated by T-Test and regression test for quantitative variables and Chi-square test for qualitative variables**.**

Results: The average FSG in diabetic and non-diabetic groups were 11.43mg/dl and 5.2mg/dl, respectively. Also the correlation coefficients between FPG and FSG in diabetic and non-diabetic groups were 0.835 and 0.583 respectively (p-value=0.0001).

Conclusion: This study showed that there is a significant linear relationship between FPG and FSG. Therefore, FSG amounts can be used as a non-invasive method to detect FPG.

Keywords:diabetes mellitus, blood, saliva, glucose

Introduction

Diabetes Mellitus (DM) is a chronic condition with increasing prevalence and incidence rates across the globe, especially in the Middle East countries (1).According to the recent reports by Diabetes International Federation (IDF), there have been about 285 million patients with DM in the world and this will reach to 438 million patients in 2030 (2).

Laboratory diagnosis of DM is based on glucose measurement in two situations: being in fasted state and not (random or causal glucose) (3).Definitive diagnosis of the DM is based on the specific laboratory findings, as well as presence of clinical signs and symptoms. The glucose level analysis is done by different methods. Fasting plasma glucose (FPG) is the most common method to measure

patients' glucose level and find out how much the disease is controlled. There are some shortcomings of this method; it's a somehow aggressive procedure that may increase patients distress. Therefore, authors aimed to investigate an alternative and simple method to measure patient's glucose level; that is the determination of Fasting Salivary Glucose (FSG).

Most of the studies have shown that FSG level in DM subjects is greater than healthy people (4-13). However, there are some controversies regarding glucose assessment via this method arising from problems such as storing offood carbohydrates in saliva (14,15), sugar consumption by mouth flora (16), carbohydrates release by salivary glycoprotein (17,18) and salivary contamination by elevated cervicular fluid in patients with gingival diseases (19,20). Investigation ofthe relationship between salivary and blood glucose levels comprising some methodological limitations and different conclusions has been brought about because of the different methodologies, various collection procedures of blood and saliva samples, and different sample volumes. Moreover, the synchronicity of blood and saliva collection and investigation of the long-term disease control by HbA1C, have not been considered. Noting the above mentioned controversies, limited information and presence of high detectable glucose in saliva of diabetics,we aimed to investigatethe relation between FPG and FSG level in diabeticand non-diabetic patients in a descriptive analytic study as we hypothesized that FSGdetermination can be used as a noninvasive method to detect FPG level.

Materials and Methods

Subjects

This was a cross-sectional study performed on 120 subjects of 25-50 year old. Patients were referred to central laboratory of Yazd, Iran by their physician in order to measure FPG and HbA1C. The study subjects were selected after taking demographic dataand medical history. Patients were informed about the aim of the study and aftertaking consensus from them all, of each; they entered in the study and were divided into 2 groups. From the total of 120 subjects, 60 patients participated as the test group and 60 as the control group. Finally, according to inclusion and exclusion criteria, 52 patients from the test group and 47 patients from the control group participated in this study. The meanage ofdiabetic andnon-diabetics group was 41.3±5.7 years and 39±6.4 years, respectively.

Inclusion criteria for the test group consisted from having positive history of established diabetes type2 i.e. GTT≥200 mg/dl, FPG≥126 mg/dl, BS≥200 mg/dl and positive cardinal signs including history of weight loss, polyuria, polyphagia, polydypsia and muscle weakness). This citeria for control group included no history of DM, and FPG lower than 126mg/dl in the last month.

Inboth groups, patients with dry mouth, Sjogren's syndrome, heart disease, pregnancy, severe periodontitis, salivadecreasing drug usage, smoking, positive history of salivary gland surgery, and history of chemotherapy or head and neck radiotherapy in the last month excluded from the study.

Sampling and Laboratory Procedures

In a fasting state at 8 AM, the blood sample was taken from both groups then they were asked to collect their whole saliva (unstimulated) in a sterile glass tube over a period of5 minutes using spiting method in which: Everypatient was asked to sit in the dental chair with head tilted forward and instructed not to speak, swallow, or do any head movements during the procedure, oreven swallow any saliva if present in the mouth. Then small quantities of NaF powder were added into salivary samples in order to suppress the glycolysis cycle and glucose consumption by salivary bacteria. Mixingdone by plastic spatula. The samples were then immediately centrifuged at the speed of 4400 rpm for 15 minutes in order to be purified. The isolated samples were transferred to plastic

tubes by pipettes; then parafinated and freezed at a temperature about-40°C. FPG level was measured by GOD-PAP method (glucose Liquicolor kit, Germany), which is an enzyme colorometric test without deproteinisation. HbA1C level was measured by immunoturbidimetric method (HbA1C quantitative measure kit, Pars Azmoon co, Iran). FSG level was measured by glucose oxidase/peroxidase method (Biosystems S.A. Costa Brava30, Barcelona, Spain) with the detection limit of about 0.23mg/dl or 0.0126mmol/L (figure1). Firstly the standard concentrations (0.325, 0.75, 1.25, 2.5, 5 and 10) were obtained, then 100 landa was separately taken from eachinto sterile glass tubes and 1000 landa reagents wereadded to all(Figure2).They were stored in incubator with temperatureof about 37°C. Then, the absorption concentration rates were read by spectrophotometer (Figure 3).Standard curve was drawn according to concentration absorption rates. Then 100 landa from each salivary sample was transferred into separated glass tubes and 1000 landa reagent was added to each. Again, the samples were stored in incubator with temperature of about 37°C and the absorption concentration rates were read by spectrophotometer and FSG concentration was evaluated (figure 1).

The standard formula for obtained concentration of salivary glucose was as follows:

$$\text{Concentration}=\frac{\text{Absorption}-0.0111}{0.0156}$$

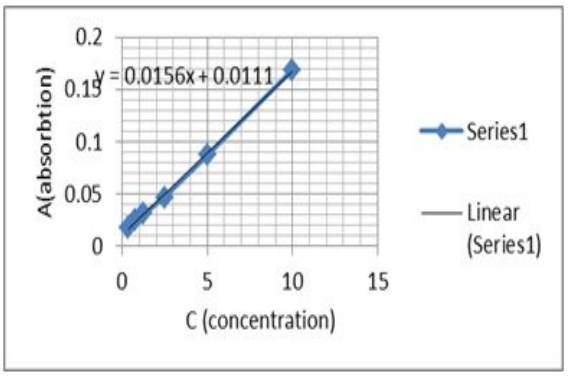

Figure 1: Standard curve for absorption rates concentration shown by spectrophotometer.

Statistical analysis

Statistical analysiswas doneby t-test regression test for quantitative variables and Chi-square test for qualitative variables, respectively. P-value less than 0.05 were considered significant**.**

Results

Demographic data of the subjects are shown in Table 1. There were no significant differences between the diabetic and non-diabetic group regard to age (p-value=0.06) and gender (p-value=0.287). As shown in Table 2 mean FPG level in diabetic group was 184.67mg/dl and in non-diabetic group was 98.42mg/dl and this difference was significant (p-value=0.0001)

The average of FSG level in diabetic group was higher than non-diabetic subjects and this difference was statistically significant by T-Test (p-value=0.0001).

Mean HbA1c level in diabetic group was significantly higher than non-diabetic subjects (p-value=0.0001)

The correlation coefficient between were0.835 and 0.583 respectively which was significant statistically (p-value=0.0001).There was a strong relationship between FSG and FPG indiabetic and non-diabetic groups (r=0.835 and 0.583, repectively; p=0.0001) (Table 3).

the correlation coefficientin non-diabetic group was -0.112 (p-value = 0.454) and in diabetic group was 0.516 (p-value=0.0001). Also, there was a strong and significant relationship between FSG and HbA1C in diabetic patients (r=0.516, p-value=0.0001);

Table 1- The comparison between mean age and gender in Diabetic and Non-Diabetic patients

Variable		Diabetic (n=52)	Non-diabetic (n=47)	p-value
Gender	**M**	21	24	0.287
	F	31	23	
Mean Age		41.3	39	0.06

Table 2- The relation between FPG and FSG in Diabetic and Non-Diabetic patients

Variable	Diabetic (n=52)	Non-diabetic (n=47)	p-value
FPG	184.67	98.42	0.0001
FSG	11.43	5.2	0.0001

but this association was not significant for no-diabetic subjects (p-value=0.454) (Table 4).
According to the correlation results in this study, a linear regression formula between the FPG and FSG was obtained as below:
In test group:

$$\mathrm{FPG} = \frac{\mathrm{FSG} + 1.7}{0.071}$$

And in control group:

$$\mathrm{FPG} = \frac{\mathrm{FSG} + 0.43}{0.057}$$

Both formulas were statistically significant (p-value=0.0001).

Discussion

This study showed that there was a significant linear relationship between FPG and FSGand the correlation coefficient between FPG and FSG level in diabetic patients was 0.835 and in non–diabetics was 0.583,and both quantities were statistically significant (p-value=0.0001).
DM is a complicated, systemic and metabolic disorder characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to metabolic action of insulin on target tissues (14). In DM, the metabolism of lipids, carbohydrates and proteins are affected. Hyperglycemia is the main feature of DM and its side effects can affect different organs (21-23).The prevalence of DM has been about 180 million patients in 2000, and it is predicted that it will reach up to 314 million till 2025 (24). This metabolic disease is a burden on both patients and society because of the high morbidity and mortality associated with infections in different organsas well asrenal, retinal, and vascular complications. Primary prevention of DM and its complications are of great practical importance (14).
As it is known,blood sample is the most common biologic fluid utilized for diagnosis and monitoring of diseases. However, whole saliva is frequently studied as an alternative for blood that can be useful even for diagnostic purposes. Whole saliva contains locally produced substances as well as serum components that can be used for diagnosis of a variety of systemic diseases and understanding their oral manifestations.Two of the advantages of salivary assessment are its non-invasive collection and cost effectiveness for screening large populations. Therefore, it has showed favorable results as an alternative method for the diagnostic purposes in various studies (24).While FPG evaluation is the most important method for diagnosisof DM, this is an aggressive and stressful method to most patients.
Instudies on diabetic patients, researchers have noticed that the FSG level had been greater in diabetic patients than non-diabetics (4-7).
In a study by Amer et al. in 2001 (1), they reported the FSG level was only detected in diabetic patients and they also revealed positive significant relation between FSG and FPG in diabetic patients and the correlation coefficient was 0.78. The reason of not detecting the glucose in saliva in non diabetic patients may be related to the type of the kit applied. The GOD-PAP kit has been applied and this kit is not sensitive in case of lower glucose level, but greater level of the glucose (greater than 20mg/dl) is detected simply. Therefore it is suitable for glucose detection in

Table 3- The relation between FSG and FPG in diabetic and non diabetic group

Group	Variable	Correlation	Sig.
Diabetic	FPG,FSG	0.835	0.0001
Non diabetic	FPG,FSG	0.583	0.0001
Statistical analysis		Pearson correlation	T-Test

Table 4- The relation between HbA1C and FSG in diabetic and non diabetic group

Group	Variable	Correlation	Sig
Diabetic	HbA1C,FSG	0.516	0.0001
Non diabetic	HbA1C,FSG	0.112	0.454
Statistical analysis		Pearson correlation	T-Test

blood samples and not in saliva as it has sensitivity to glucose concentration lower than 20mg/dl. We applied oxidase/peroxidase kit in our study which is capable of glucose detection in lower concentrations even in non-diabetics (sensitivity range= 0.23mg/dl).

In Hashempour et al. study in 2001 (6), the relationship between FPG and FSG level in non-diabetic patients was evaluated. FSG was evaluated by oxidase glucose, this study revealed that the correlation coefficients between FSG and FPG were about 0.05 to 0.87 which showed a weak relation in some and strong relation in other but finally they concluded that the FSG can not be a criterion to evaluate FPG. This may be resulted from insufficient sample.

Thorestenson et al. in 1989 (4) also showed that FPG level in diabetic patients is greater than non-diabetics but they could not reveal any relation between FPG and FSG.

In the study by Belazi et al. in 1998 (5), in 10 diabetic (IDDM) and 10 non-diabetic infants, there was not a positive relation between FSG and FPG though theirconcentrationswere significantly greater in diabetics than non-diabetics.

In another study by Aydin et al. in 2006 (9), FSG was evaluated by glucose oxidase method in 40 healthy and 20 diabetic patients. They mixed 200 landa of saliva with 1000 landa of reagent.Although FSG concentration in diabetic patients was greater, the difference was not significant. This may be due to the insufficient sample volume and less sensitivity of the kit to detect FSG; but we applied 100 landa instead of 200 which showed high sensitivity of the kit.

Sashikumar et al. in 2010 (14) added 10 landa salivary sample to the 1000 landa of the reagent. However, they also reported that the FSG in diabetic patients was greater than non-diabetics but there was no significant relation between FSG and FPG probably because of low saliva volume.

In Vaziri et al. study in 2009 (24) although FSG in diabetic subjects was greater than normal subjects,they did not observe any relation between FSG and FPG.In this study the glucose was evaluated by glucose oxidase method. This technique has lesssensitivity to lower level of glucose and could not detect the lower glucose concentrations in saliva.

Like the current study,Jurysta etal.in 2009 (25)concluded that in diabetic patients, as compared to control subjects, the relative increase in saliva glucose concentration was comparable.The relationship between these two variables was also documented in normal subjects and diabetic patients undergoing an oral glucose tolerance test,despite completely different methods .

In Ana Carolina study in 2010 (26), they concluded that salivary glucose concentration was significantly higher in the diabetic group and that there was no correlation between salivary and blood glucose concentrations in diabetic patients. It is believed that the difference of results is due to different methods utilized although the kits for salivary glucose were the same.

In Hallikerimath study in 2011 (27) they reportedthe highly sensitive test procedures that are now commonplace make it practical to quantitate, despite very low concentrations a large number of hormones and drugs in saliva. Tests based on saliva have already made substantial inroads into diagnosis. For some molecules for example, antibodies, unconjugated steroids, hormones and certain drugs the techniques are sufficiently sensitive to reflect blood concentrations of the substance accurately. The following study explores the possibility of using saliva to reflect the glucose concentration in blood, thereby making self-measurement of glucose less invasive.

In Ivanovski study in 2012 (28) they claimed that Salivaryglucose was determined by using the enzymatic method with a hexokinase. Varying degrees of xerostomia were noticed in 80% of the experimental group and only 10% of the control group. In diabetics, they found significantly higher levels of glucose (0.022 mmol/l) in the saliva compared with their values in the control group (1.48 mmol/l,

0.017mmol/l). Based on these results, they concluded that diabetes is a disease that causes xerostomia and there is a significant correlation between the degree of xerostomia and the salivary level of glucose.These measures was close to what we achived in our survey.

This study also showed that there is a strong correlation between HbA1C and FSG in diabetic group (r=0.516, p=0.0001).In non-diabetics, however, this association was notstatistically significant (r =-0.112, p value= 0.454).

As theHbA1C measure is a key to find out the patient's diabetes control status,and because (according to current study results) the mean HbA1c level was higher in diabetics than non-diabetics, we suggest salivary glucose evaluation as an alternative method to evaluate diabetes control.

This study also showed a linear regression formula according to the presence of correlation between fasting salivary and blood glucosefor the first time and FSG determination can be used as a non-invasive method to detect FPGand DM situation .

Aknowdelgements

We would like to express our appreciation to Research Deputy of Shahid Sadoughi University of Medical Science of Yazd, Iran. We would like to thank them for the financial support and also Abu-Ali Laboratory in Yazd, Iran. Special thanks to Dr Hakime Ahadian for her collaboration.

This paper is extracted frompart of a student thesis proposal.

References

1. Amer S, Yousuf MUNI, Siddqiui PQ, Alam JUNA. Salivary glucose concentrations in patients with diabetes mellitus a minimally invasive technique for monitoring blood glucose levels. Pakistan journal of pharmaceutical sciences 2001;14(1):33-7.
2. Raha S,Akhavan karbasi M.H. A relation comparison between blood sugar of the tooth sucket and the side effects after dental extraction in two groups of type2 diabetics and non diabetic patients. Resarch proposal in dental school of shahid sadoughi university of medical science,Yazd,Iran, 2010;398:1
3. American Diabetes Association. Exclusive summary Standards of medical care in diabetes. Diabetes care 2011;34(1):4-10
4. Thorstensson H, Falk H, Hugoson A, Olsson J. Some salivary factors in insulin-dependent diabetics. Acta Odontologica 1989;47(3):175-83.
5. Belazi MA, Galli-Tsinopoulou A, Drakoulakos D, Fleva A, Papanayiotou PH. Salivary alterations in insulin-dependent diabetes mellitus. International Journal of Paediatric Dentistry 2002;8(1):29-33.
6. Hashempour M, Nikuee F, Amini M, Amioreaya A, Rezvanian H. Investigation of the relation between blood sugar and salivary glucose to find a non-aggressive method to measure blood sugar. J Iranian metabolism 20012;(4):221-6
7. Hase JC, Birkhed D. Oral sugar clearance in elderly people with prosthodontic reconstructions. European Journal of Oral Sciences 1991;99(4):333-9.
8. Hase JC, Birkhed D, Laqerlof F, Thornqvist E. Oral retention of glucose at pharmacologically reduced salivary flow in man. Scand J Dent Res 1994;102(3):180-5
9. Aydin S. A comparison of Gherlin, Glucose, Alpha- amylase and protein levels in saliva from diabetes. J Biochem Mol Biol 2007;40(1):29-35
10. Leach SA, Critchley SA. Bacterial degradation of glycoprotein sugars in human saliva. Nature 1966;209(5022):506
11. Leach SA, Melville TH. Investigation of some human oral organisms capable of releasing carbohydrates from salivary glycoproteins. Arch Oral Biol 1970;15(1):87-8
12. Kjellman O. The presence of glcouse in gingival exudates and resting saliva of subjects with insulin treated diabetes mellitus. Sven Tandlak Tidskr 1970;63(1):11-9
13. Ficara AJ, Levin MP, Grower MF, Kramer GD. A comparison of glucose and protein content of gingival fluid from diabetics and non-diabetics. J Periodontal Res 1975;10(3):171-175
14. Sashikumar R, Kannan R. Salivary glucose levels and oral candidal carriage in type II diabetics. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2010;109(5): 706–11

15. Karjalainen KM, Knuuttila ML, Käär ML. Salivary factors in children and adolescents with insulin-dependent diabetes mellitus. Pediatr Dent 1996;18(4):306-11.
16. Schneider H, Shaw J, Zimmet P. Guidelines for the detection of diabetes mellitus diagnostic criteria and rationale for screening. Clin Biochem Rev 2003;24(3):77-80.
17. Vernillo AT. Diabetes mellitus: Relevance to dental treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91(3):263-70.
18. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26(1):5-20.
19. Kaufman E, Lamster IB. The diagnostic applications of saliva:a review. Crit Rev Oral Biol Med 2002;13(2):197-212
20. Kimura I, Sasamoto H, Sasamura T, Sugihara Y, Ohgaku S, Kobayashi M. Reduction of incretinlikesalivatin in saliva from patients with type 2 diabetes and in parotid glands of streptozotocindiabetic BALB/c mice. Diabetes ObesMetab 2001;3(4):254-8.
21. Yamagushi M, Mitsumuri M, Kano Y. Noninvasively measuring blood glucose using saliva: IEEE Eng med boil 1998;17(3):59-63
22. Shanon IC. Blood and saliva glucose level in relation to gingival health. J Indiana Dental Assoc 1973;45(10):299-302
23. Ginberg BH. An overview of minimally invasive technologies. Clinical Chem 1992;38(9):1596-600
24. BakianianVziri P, Vahedi M, Mortazavi H, Abdollahzadeh Sh, Hajilooi M. Evaluation of salivary Glucose, Iga and flow rate in diabetic patients: A case control study. J Dent Tehran 2010;7(1):13-8
25. Jurysta C, Bulur N, Oguzhan B, Satman I, Yilmaz TM, Malaisse WJ, et al. Salivary glucose concentration and excretion in normal and diabetic subjects. BioMed Research International 2009;2009.
26. Vasconcelos AC, Soares MS, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. Journal of oral science 2010;52(2):293-8.
27. Hallikerimath S. Study of serum and salivary glucose levels in type 2 diabetic patients. 2011,http://hdl.handle.net/123456789/161
28. IvanovskiK, Naumovski V, Kostadinova M, Pesevska S, Drijanska K, Filipce V. Xerostomia and salivary levels of glucose and urea in patients with diabetes. Prilozi Macedonian Academy of Sciences and Arts, Section of Biological and Medical Sciences 2012;33(2):219.