

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

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### Abstract

**Objective:** Fasting plasma glucose (FPG) analysis is the most important method 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 Glucose as a simple and non-invasive method to evaluate FPG has come into significant consideration of 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 FSG level 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 11.43 mg/dl and 5.2 mg/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 casual 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 of food carbohydrates in saliva (14,15), sugar consumption by mouth flora (16), carbohydrates release by salivary glycoprotein (17,18) and salivary contamination by elevated cervical fluid in patients with gingival diseases (19,20). Investigation of the 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 investigate the relation between FPG and FSG level in diabetic and non-diabetic patients in a descriptive analytic study as we hypothesized that FSG determination 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 data and medical history. Patients were informed about the aim of the study and after taking 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 mean age of diabetic and non-diabetic group was  $41.3 \pm 5.7$  years and  $39 \pm 6.4$  years, respectively.

Inclusion criteria for the test group consisted from having positive history of established diabetes type 2 i.e.  $GTT \geq 200$  mg/dl,  $FPG \geq 126$  mg/dl,  $BS \geq 200$  mg/dl and positive cardinal signs including history of weight loss, polyuria, polyphagia, polydipsia and muscle weakness). This criteria for control group included no history of DM, and FPG lower than 126mg/dl in the last month.

In both groups, patients with dry mouth, Sjogren's syndrome, heart disease, pregnancy, severe periodontitis, saliva decreasing 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 of 5 minutes using spitting method in which: Every patient 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, or even 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. Mixing done 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 each into sterile glass tubes and 1000 landa reagents were added to all (Figure2). They were stored in incubator with temperature of 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:

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

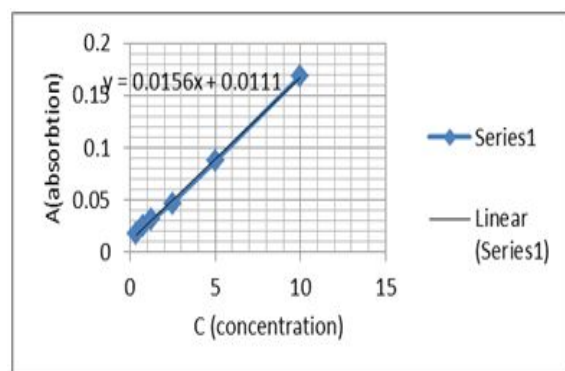


Figure 1: Standard curve for absorption rates concentration shown by spectrophotometer.

## Statistical analysis

Statistical analysis was done by 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 were 0.835 and 0.583 respectively which was significant statistically (p-value=0.0001). There was a strong relationship between FSG and FPG in diabetic and non-diabetic groups (r=0.835 and 0.583, respectively; p=0.0001) (Table 3).

the correlation coefficient in 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

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but this association was not significant for non-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:

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

And in control group:

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

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

### Discussion

This study showed that there was a significant linear relationship between FPG and FSG and 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 organs as well as renal, 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 diagnosis of DM, this is an aggressive and stressful method to most patients.

In studies 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 their concentrations were 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 less sensitivity to lower level of glucose and could not detect the lower glucose concentrations in saliva.

Like the current study, Jurysta et al. 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 reported the 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 Salivary glucose 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 achieved 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 not statistically significant ( $r=-0.112$ ,  $p$  value= $0.454$ ).

As the HbA1C 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.

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