

Correlations between Fasting Salivary Glucose and Fasting Plasma Glucose in Type 2 Diabetes Mellitus

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Abstract :Background : Early detection & screening of type 2 Diabetes Mellitus (DM) is essential to delay its complications. However, testing for DM often requires invasive and painful blood testing which deters the people from undergoing an investigation and they remain undetected for a very long time. So, there is a need for non-invasive testing.

Aim : This study aimed at estimating and correlating fasting salivary glucose and fasting plasma glucose in type 2 diabetics and non-diabetics; so as to assess if salivary glucose can be used as a non-invasive tool, to evaluate blood glucose levels, and could it also be used as a biomarker for large scale screening of diabetes.

Method : 50 type 2 diabetic and 50 non-diabetic participants were selected according to inclusion & exclusion criteria. Quantitative estimation of blood and salivary glucose were performed by glucose oxidase method. Pearson's correlation coefficient test was applied to assess the correlation between salivary glucose level and plasma glucose level.

Result : The mean salivary glucose was significantly greater in type 2 diabetics (1.45 ± 0.91 mg/dL) as compared to non-diabetic participants (0.41 ± 0.29 mg/dL). A positive, significant but moderate correlation was seen in both type-2 DM patients ($r = 0.43$; $p = 0.002$) and in non-diabetics ($r = 0.44$; $p = 0.001$). The sensitivity and specificity were 66% and 96% at 1mg/dl respectively.

Conclusion : Fasting salivary glucose could potentially be used as a non-invasive tool for monitoring and for large scale screening of type 2 DM.

Keywords: Diabetes Mellitus, Non-invasive, Plasma Glucose, Salivary Glucose.

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I. Introduction

Diabetes Mellitus, the most common metabolic disorders with chronic hyperglycemia over a prolonged period.^[1] According to International Diabetes Federation in the year 2015, about 415 million people were suffering with DM worldwide. Type- 2 diabetes comprises about 90% of the diabetic population. In the early stage of type 2 DM, people are asymptomatic; therefore, they are not detected early.^[2] The average time lag between the actual onset and the diagnosis of type 2 diabetes is 7 years. This delay in diagnosis is the main reason for its profound complications.^[3] DM and its complications place a large financial burden on individuals and health care system. The number of work hours lost also increases due to its morbidity. So, type 2 DM which is considered as an iceberg disease is exponentially increasing in its incidence, prevalence, complications, morbidity and mortality.^[2]

The principle detection method of DM requires a venepuncture or a painful needle prick, this deters the people from undergoing an investigation and thus they go undetected for a very long time.^[4] This invasive investigation also results in poor compliance in patients, resulting in increasing complications associated with DM. Repeated monitoring of blood glucose levels in diabetes is painful, inconvenient and costly.^[5] Furthermore, blood collection by venepuncture requires trained persons and handling blood samples is not safe. So there is a need to find some non- invasive means to evaluate blood glucose levels frequently without any discomfort to the patient.^[3, 6]

Currently, urine and blood tests are available for screening type 2 DM. However, urine tests have several drawbacks. Namely, the increase in blood sugar levels needs to be large to be detected in urine. Since urine accumulates over time, it is more difficult to collect under fasting conditions as compared to blood. Collection of urine sample is also messy and inconvenient. And unfortunately the correlation between urine and plasma glucose was inconsistent.^[7-9]

Some noninvasive or minimally invasive techniques for blood glucose monitoring were studied, including infrared (IR) spectroscopy, Raman spectroscopy, fluorescence spectroscopy, occlusion spectroscopy

and surface plasmon resonance. Nevertheless, the results still have to be correlated with direct blood glucose measurements; the sensitivity and reliability are limited by skin thickness and spectral signal-to-noise level.^[9]

Taking into considerations the gravity of the situations concerning DM and the drawbacks of blood, urine and other investigations for blood glucose; there is a need for another convenient, simpler, safer, more socially acceptable and non-invasive procedure to estimate blood glucose.

The advantages of saliva over blood is the relatively easy and non-invasive nature of sample collection by spitting or drooling method which is neither painful nor traumatic. It can be readily collected from the participants with modest instruction and by persons with minimal training as opposed to blood collection. Since its easy availability and hassle-free collection, it can be collected multiple times without imposing too much discomfort on the subject. No special equipment is needed to collect saliva by spitting or drooling method. It is easy to collect, transport, store, preserve and dispose saliva. There is minimal risk of acquiring infections; thus, it is safer for the hospital staff to handle saliva as compared to blood and other body fluids. It can be used in clinically challenging situations when blood sampling is difficult and when compliance is a problem; such as in infants, children, handicapped, mentally retarded, uncooperative or anxious patients. It is an inexpensive method as well. Thus saliva offers advantages over serum which would highly improve compliance and glucose control while reducing DM complications and overall disease management costs.^[4, 7, 9-15]

Saliva is a complex oral fluid that includes secretions from major and minor salivary glands. The three pairs of major salivary glands include parotid, maxillary and sublingual glands. It also includes secretion from the minor salivary glands of the labial, buccal, lingual, palatoglossal salivary glands and palatal mucosa. Based on the source, saliva can be classified as Whole saliva or Gland-specific saliva. Whole saliva can be collected by draining/drooling method in which saliva is allowed to drip off the lower lip. Another method is spitting method, in which the subject spits saliva into a test tube/ container. In soaking method, the subject holds or chews a sterile cotton swab in the mouth; which when soaked is squeezed by a syringe with a membrane to obtain saliva. Based stimulation, saliva can be classified as Un-stimulated saliva or Stimulated saliva which is collected after mechanical or chemical stimulation. Saliva is said to be an ultra-filtrate of blood and can be used as a diagnostic medium. Though saliva may functionally be equivalent to serum, it contains components in concentrations that are 100 to 3000 fold less than those in blood. However nowadays highly sensitive detection techniques are available. Therefore, we can unveil the utility of saliva as a diagnostic medium despite its very low concentrations as compared to blood.^[6, 9-12, 14, 16, 17]

Studies have shown raised levels salivary glucose level in diabetes which can be detected colorimetrically by automated or semi automated analyzers by Glucose oxidase –Peroxidase method.^[3, 4, 6, 7, 18, 19]

But there is conflict among some of the studies about correlations between salivary and blood glucose levels.^[3,4,6-8,18]

So this study aims at correlating salivary glucose and blood glucose levels in type 2 DM and non-diabetic controls; and thus explores the possibility of using saliva to reflect the glucose concentration in blood, thereby making evaluation & screening of blood glucose less invasive.

II. Material & Methods

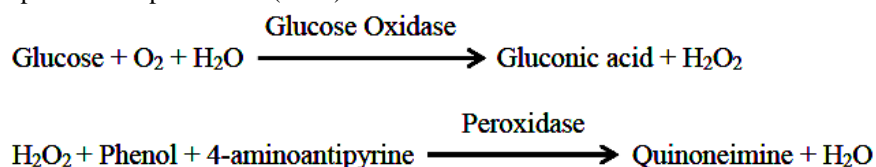
The present study is a comparative, cross-sectional study. A protocol was designed and approved by the Institutional Ethical Committee. 100 study participants were grouped into two groups. 50 already diagnosed type 2 Diabetics were enrolled in Group 1 and 50 non-diabetics were enlisted in Group 2 according to inclusion and exclusion criteria. Known cases of type 2 Diabetes Mellitus whose fasting plasma glucose was >126mg/dl and/or 2 hr plasma glucose levels >200mg/dl, with duration ≤ 10 years, of the ages between 35-70 years and of both sexes were included in Group 1. Participants with any oral or dental pathology at the time of study, pathology that could affect salivary gland functions like parotitis or salivary gland surgery, participants with history of alcohol consumption, tobacco consumption or smoking, participants with a history of radiotherapy for head and neck cancer, or having any other chronic medical / systemic illness other than type 2 diabetes and hypertension were excluded from the study. Non-diabetic participants whose fasting plasma glucose was < 110mg/dl and 2 hr plasma glucose levels <140mg/dl were included in the study.

Saliva collection & estimation: The participants were advised to come fasting, that is foods and fluids (except water) should not be taken for at least 8 hours or overnight before the test. They were advised to avoid brushing for at least 2 hours prior to saliva sample collection. Complete procedure was explained to the participants and an informed written consent was taken. A detailed history was taken. The participants were told to relax and to sit comfortably. Then whole & un-stimulated saliva was collected in a sterile plastic container over 10 minutes by spitting method. The samples were processed and analysed immediately. The saliva sample was transferred from the sterile plastic container to test tube and centrifuged at 3000 rotations per minute for 15 minutes in order to remove mucin & other sediments such as oral epithelial cells, any micro-organisms, food debris etc., and the clear supernatant obtained after centrifuging was immediately processed for estimation of glucose. 10 µL of saliva sample was mixed with 1ml of GOD-POD enzyme reagent and incubated for 15 min at 37°C and then

absorbance values were measured colorimetrically in a semi-auto-analyser. For salivary glucose estimation the semi-auto analyser was standardized at 10mg/dL.

The plasma glucose level was measured using the Glucose Oxidase-Per Oxidase (GOD-POD) method in a semi-automated analyser which was standardized at 100mg/dL.

Principle of Glucose estimation: Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H₂O₂) is detected by a chromogenic oxygen acceptor phenol, 4-aminophenazone in the presence of peroxidase (POD).



Quinoneimine, a pink-red colored complex formed will be measured colorimetrically and the intensity of the colour formed will be directly proportional to the concentration of glucose in the sample. [6]

Statistics: Unpaired t- test for statistical significance was applied between cases and controls for Fasting Plasma Glucose levels and Fasting Salivary Glucose levels. Pearson’s correlation test was used to correlate between salivary glucose levels and plasma glucose levels in both type 2 diabetics and healthy controls in SPSS version 20.

III. Results & Observations

There were 23 males and 27 females in the type 2 DM group while there were 21 males and 29 females in the non-diabetic group. The mean age was 52 ± 8.5 years and 50.3 ± 9.17 years in type 2 DM group and non-diabetic group respectively. The average duration of diabetes was 5.7 ± 3.03 years.

Table 1: Fasting Glucose levels in the study groups.

Parameter	Type 2 DM	Non-diabetics	Unpaired t – test	
	Mean ± SD	Mean ± SD	p - value	t - value
FPG (mg/dL)	232.8 ± 81.68	98.1 ± 16.92	< 0.00001	11.41756
FSG (mg/dL)	1.45 ± 0.91	0.41 ± 0.29	< 0.00001	7.76161

FPG: Fasting Plasma Glucose;FSG: Fasting Salivary Glucose

SD: Standard Deviation; p value ≤ 0.05 was considered as significant at 95% confidence interval. The mean FPG in the Type 2 DM group is 232.8 ± 81.68 mg/dL, within a range of 117 – 411 mg/dL. The mean FPG in the non-diabetics group is 98.1 ± 16.92 mg/dL, within a range of 68 – 125 mg/dL. The mean FSG in Type 2 DM is 1.45 ± 0.91 mg/dL, ranged within 0.39 – 4.56 mg/dL. The mean FSG in the non-diabetics group is 0.41 ± 0.29 mg/dL, ranged within 0.04 – 1.37 mg/dL.

The Pearson’s correlation between FPG levels and FSG levels in type 2 DM group is statistically significant with a moderate correlation r = 0.43, p = 0.002 which is shown in Fig 1. Likewise, the correlation between FPG levels and FSG levels in the non-diabetic group is statistically significant with a moderate correlation r = 0.44, p = 0.001 which is shown in Fig 2.

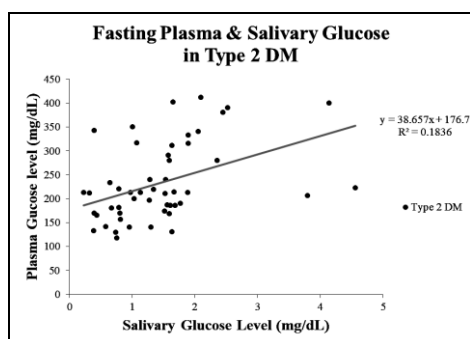


Fig 1: FPG & FSG in type 2 DM

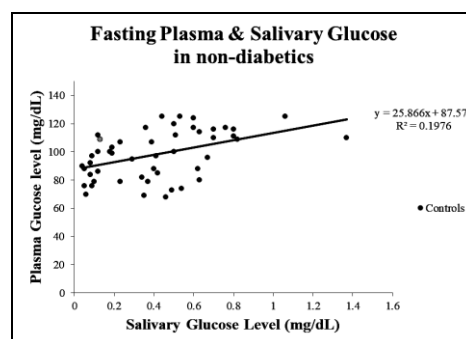


Fig 2: FPG & FSG in non-diabetics

IV. DISCUSSION

For salivary glucose levels, the results of the present study are in accordance with most previous studies. [3, 4, 8, 10, 13, 20, 21]

There are certain studies whose mean salivary glucose levels are much higher than this study. [4, 6, 11, 14-17, 19, 22-25] The variability in the results may be a reflection of different population samples, different sampling techniques for collecting saliva, and different reagents, ratios & methods used for analysis of salivary glucose. [8]

Similar to the present study, most of the studies had obtained statistically significant higher levels for salivary glucose in diabetics as compared to non-diabetics. [3, 4, 6, 7, 10-12, 14-20, 24-29]

About the strength of correlation between salivary glucose and blood glucose, a few studies found almost no correlation between salivary glucose and blood glucose. [8, 18, 23, 25]

Similar to this present study, many studies found a positive moderate correlation between salivary glucose and blood glucose. [4, 6, 7, 12, 14, 16, 25, 29]

There were some studies with stronger positive correlation. [3, 10, 12, 13, 15, 20, 22, 24, 30]

Pathophysiology of Glucose in Saliva: Though the mechanism of secretion of glucose is still obscure, both paracellular and intercellular pathways have been proposed. Sreedevi et al quoting the works of Harrison commented that glucose is a small molecule that easily diffuses through semipermeable membranes. Thus, large amounts of glucose become available in saliva when blood glucose levels are elevated, as in diabetes. [13, 17, 20]

Panchabhai AS et al and Dhanya M suggested that saliva is an ultra-filtrate of blood & glucose is one of the blood components that is transferable across the salivary gland epithelium in proportion to its concentration in blood. [4, 10]

Ramaya et al proposed that glucose diffuses easily through endothelium of blood vessels, passes through interstitial fluid to gingival fluid by way of gingival sulcus (a potential space between the teeth and the gums) and makes its way into whole saliva. [29] So, glucose in whole saliva may not be entirely derived from salivary glands alone but also from gingival crevicular fluids and oral mucosa. [13, 20]

Panda Abhikshyeet et al and RM Gali et al suggested that hyperglycaemia leads to gland impairment and its dysfunction. Hyperglycaemia leads to increased "Advanced Glycosylation End products" (AGE) which crosslink some proteins such as collagen and extracellular matrix proteins; this leads to basement membrane alteration and endothelial dysfunction. This alters the microvasculature structure and makes it more permeable. Other products formed because of chronic hyperglycaemia, such as sorbitol, diacylglycerol, and fructose-6-phosphate, also lead to basement membrane alteration. The end result is a leaky microvasculature and a leaky basement membrane, which explains the increased passage of glucose from the blood into the saliva in diabetes mellitus. [13, 16]

Reduced salivary flow rates in diabetic patients as compared to non-diabetic individuals could avoid dilution of glucose in saliva and so may increase salivary glucose concentration. [26] Thus, the presence of glucose in whole saliva is multifactorial.

The limitation of this study is that the diabetic participants were not classified into well controlled and poorly controlled diabetics; this would have given a clearer picture. Random and post prandial salivary glucose correlations with respective plasma glucose were not included in this study. Many factors influence the secretion of saliva and its constituents. The sample size was not large enough to fortify the findings. Hence, the technique adopted in this study requires validation in large number of patients.

According to this study, although salivary glucose per se may not show enough consistency to be used as an independent and autonomous biomarker for type 2 DM; in the future it can be combined with other salivary biomarkers to create an effective evaluation of blood glucose and a highly sensitive & specific large-scale screening system for type 2 DM.

V. CONCLUSION

According to the results in this study, there is a positive, significant but moderate correlations between salivary glucose levels and plasma glucose levels, in both type-2 DM patients and in non-diabetics. Therefore, salivary glucose estimation could potentially be used as a non-invasive tool for blood glucose evaluation and for large scale screening for type 2 DM. Furthermore, for detection of salivary glucose, more sensitive, refined and portable technology like a salivary glucometer should be sought for, to unveil the non-invasive potential of saliva for detection, monitoring and screening of Diabetes Mellitus.

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