

Glucose Level Estimation in Diabetes Mellitus By Saliva: A Bloodless Revolution

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Background. Diabetes mellitus is a massive, growing, silent epidemic that has the potential to cripple health services in all parts of the world. Currently, a diagnosis of diabetes is achieved by evaluating plasma glucose levels. Saliva offers some distinctive advantages. Whole saliva can be collected non-invasively and by individuals with limited training. The present study was aimed to estimate and correlate the plasma and salivary glucose levels in diabetic and non diabetic subjects, with special reference to age.

Method. The study population consisted of three groups: Group 1 consisted of diabetics with BGL>200mg/dl and Group 2 consisted of diabetics with BGL 130-200mg/dl based on their random plasma glucose levels. Group 3 consisted of healthy population as controls with BGL <130 mg/dl. 2 ml of peripheral blood was collected for the estimation of random plasma glucose levels and unstimulated saliva was collected for the estimation of salivary glucose

Results. The salivary glucose levels were significantly higher in group 1 and group 2 diabetics when compared with controls. The salivary glucose levels show a significant correlation with plasma glucose levels between study populations, suggesting that salivary glucose levels can be used as a monitoring tool for predicting glucose level in diabetic patients.

Conclusion. The present study found that estimation of salivary glucose levels can be used as a noninvasive, painless technique for the measurement of diabetic status of a patient in a dental set up.

Key words: glucose, diabetes mellitus, saliva, noninvasive.

INTRODUCTION

Diabetes mellitus is a massive, growing, silent epidemic that has the potential to cripple health services in all parts of the world. Diabetes mellitus is a group of chronic diseases characterized by insulin deficiency, cellular resistance to insulin action, or both, resulting in hyperglycemia and other related metabolic disturbances. The disease is associated with serious complications of the eyes, kidneys, heart and blood vessels, and other organ systems, which may markedly impair quality of life and shorten the patient's lifespan. Many people are affected by diabetes worldwide and the number is climbing steeply [1]. Currently, a diagnosis and monitoring of diabetes is achieved by evaluating plasma glucose levels. Blood is the most commonly used sample for laboratory diagnostic procedures, but it requires frequent invasion to vessels to collect the sample and it also causes unnecessary discomfort and mental trauma to patients; therefore, a much simpler and noninvasive technique for the diagnosis and monitoring of diabetes is very desirable [2]. Saliva offers some distinctive advantages.

Whole saliva can be collected non-invasively and by individuals with limited training. No special equipment is needed for collection of the fluid. Diagnosis of disease *via* the analysis of saliva is potentially valuable for children and older adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Further analysis of saliva may provide a cost-effective approach for the screening of large populations. The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland-specific pathology, i.e. infection and obstruction. However, whole saliva is most frequently studied when salivary analysis is used for the evaluation of systemic disorders. The best two ways to collect whole saliva are the draining method in which saliva is allowed to drip off the lower lip. Another one is spitting method in which the subject spits saliva into a test tube. Different reagents are used to determine the content of saliva [3, 4].

In the literature, we encounter a controversy regarding the relationship between the concentration of plasma glucose and salivary glucose.

Several authors show that an increase in the concentration of glucose in saliva has to do with glycaemia; however, this relationship is not confirmed in other studies [5, 6]. Some other studies have demonstrated the correlation of salivary glucose level (SGL) and blood glucose level (BGL) in diabetes [2]. The present study was aimed to estimate the correlation of plasma and salivary glucose levels in diabetic and non diabetic subjects, with special reference to age.

MATERIAL AND METHODS

The study was carried out in 3 sets of samples in total 90 patients depending on their blood glucose level (BGL). In each group contains, group I (more than 200 mg/dl of BGL), group II (more than 130-200 mg/dl of BGL), and group III (below 130 mg/dl of BGL) is healthy non-diabetic group, formed of age and gender matched healthy non-diabetic subjects. Here we group I and II considered as cases study population and group III as control population.

Written consent was obtained from each individual taking part in the study and a data sheet was completed detailing the person's name, age, sex, and relevant medical history. The cases and

control subjects were asked to come into the clinic in the morning between 8 am to 10 am, 2 mL venous blood was collected, in an ethylene diamine tetra acetic acid (EDTA) containing blood collection tube and stored. The patients were asked to wash their mouths with tap water and to spit two or three times, after which they were told to spit the saliva pooled in their mouths for the following 10 minutes/ volume of saliva into the sterile sample collection container to calculate the salivary flow rate in the case study population. The quantitative estimation of FPG, fasting plasma glucose and FSG, fasting saliva glucose was done by glucose oxidase method using enzymatic kit GOD-POD, glucose oxidase peroxidase.

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

Glucose Oxidase

Glucose+O₂+H₂O Gluconic acid +H₂O₂

Peroxidase

H₂O₂+Phenol+4-aminoantipyrine Quinoneimine +H₂O

RESULTS

Table 1

Comparison of salivary glucose between cases and control groups (Student t test)

Study population	Mean	SD	t value
Cases	12.07	1.15	-9.0 p <0.001

Table 2

Gender wise and age wise distribution of study subjects in 2 study sample sets groups

Study population groups	Gender wise distribution			Age wise distribution
	No	Male%	Female %	Mean / SD/ Range
Group III (<130 mg/dl)	37	22	15	41.08/ 15.65/ 19-88
Group II (130-200 mg/dl)	32	15	17	52.65/ 13.08 / 27-75
Group I (>200 mg/dl)	21	11	10	53.71 /7.75 /30-66
Total	90	48	42	X ² = 97.43, p< 0.67
	X ² =1.10, p < 0.57			

Table 3
Comparison of blood glucose level and salivary glucose level in study population (Anova –one way test)

BGL	Mean SGL	SD	f value
Group III (<130 mg/dl)	5.78	1.15	106.26 p <0.001
Group II (130-200 mg/dl)	9.81	2.11	
Group I (>200 mg/dl)	15.5	4.09	

Table 1 shows comparison of salivary glucose between cases and control groups. The mean value of salivary glucose level cases was 12.07 and for control group it was 5.78. A statistically significant difference has been observed between cases and control groups ($p < 0.001$, HS).

Table 2 shows comparison of salivary glucose between Gender wise and age wise distribution of study subjects. 37 subjects were found in group III (22 male and 15 female), the mean age was 41.08. 32 subjects were found in group II (15 male and 17 female), mean age was 52.65. 21 subjects were found in group I (11 male and 10 female), mean age was 53.71. However, no statistically significant difference has been observed Gender wise and age wise distribution of study subjects ($p < 0.57$ for gender wise distribution and $p < 0.67$ for age wise distribution, NS).

Table 3 shows comparison of plasma glucose level and salivary glucose level in study population. In Group III subjects mean SGL was 5.78. In group II mean SGL was 9.81 and in group I mean SGL was 15.5. A statistically significant difference has been observed between plasma glucose level and salivary glucose level in study population ($p < 0.001$, HS).

DISCUSSION

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 plasma components that can be used for diagnosis of a variety of systemic diseases and understanding of their oral manifestations. Two of the advantages of salivary assessment are its non-invasive collection and cost effectiveness for screening large populations [7, 8]. Mata *et al.* reported alterations of salivary composition in diabetic patients. These biologic changes in diabetic whole saliva were different from one study to another that may be due to the diversity in sample selection criteria and study design [9-11].

Diabetes is a globally wide spread disease. It is a group of metabolic disorders that share the common underlying feature of hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action. Chronic hyperglycemia and the attendant metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels [1]. Various diagnostic devices are available in the market to measure the blood glucose level. However, in available products blood is taken as diagnostic body fluid. There a necessity arises to establish a noninvasive procedure to determine the blood glucose level without taking blood. Keeping the above in view, the present study was aimed at estimation of blood and salivary glucose level in diabetic and non diabetic subjects. The present attempt was designed to establish a noninvasive procedure to measure glucose level using saliva which is most easily obtainable. A correlation was observed between the salivary glucose level of diabetic and non diabetic study population. These values of correlation proven by the Student t test ($p < 0.001$ Highly Significant) shown in Table 1. These results were corresponding to study done by Panda Abikshyeet *et al.* [1] and Syed Shahbaz *et al.* [11]. There was no correlation were found in Gender wise ($X^2 = 1.10$, $p < 0.57$) and age wise ($X^2 = 97.43$, $p < 0.67$) distribution of study subjects in different study sample groups shown in Table 3. The results were in concordance with the studies conducted by Panchbhair *et al.* [2].

In the present study the comparison between blood glucose level and salivary glucose level in study population (Anova – one way test) shows Group III (< 130 mg/dl BGL) mean SGL - 5.78, SD 1.15, Group II (130-200 mg/dl BGL) mean SGL - 9.81, SD 2.11 and for group I (> 200 mg/dl BGL) mean SGL - 15.5 and SD was 4.09. A statistically significant difference has been observed between blood glucose level and salivary glucose level (f - value 106.26 $p < 0.001$). We divided the patient group into three subgroups based on their plasma glucose level and compared the salivary glucose levels of those three groups. We found a statistically significant difference among the three groups, and the salivary glucose levels were found

to increase as plasma glucose levels increased. As such we can suggest that on the basis of our study the normal range blood glucose level can correlate with 4-6 mg/dl salivary glucose level. Our results were in agreement with the studies conducted by Panda Abikshyeet *et al.* [1], Satish Kumar *et al.* [10], B Fabre *et al.* [12] and Maria-Sueli-Marques Soares [5]. No correlation were found in the study conducted Panchbhai *et al.* [2, 5]. This difference could be due to the differences in method practiced by different study population group, selection criteria and also the method of collection of sample.

Saliva indeed is a mirror of our blood as these bio fluids and their molecular components share many similarities. Realization of this fact and the possible utility of saliva as a diagnostic bio fluid using recent technological advances over the past decades has enabled many researchers to develop saliva based technology to detect the transition between health and disease [11]. As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected noninvasively by individuals with modest training. Furthermore, saliva may provide a cost effective approach for the screening of a large population. It is said that "saliva lacks the drama of blood, sincerity of sweat and emotional appearance of tears" but still the fact is that, it is the vital element that sustains life in the oral cavity [13]. The present attempt was designed to establish a non-invasive procedure to measure glucose level using saliva which is most easily obtainable. We know that glucose is present in the saliva of normal individuals; however, the mechanism of its secretion is still obscure. Both paracellular and intercellular pathways have been proposed, but this is still an hypothesis rather than an established theory. Many authors have tried to explain the increased glucose content in the salivary secretion of diabetic patients. Some studies tried to show that the salivary glands act as filters of blood glucose that are altered by hormonal or neural regulation [1, 3, 14]. Persistent hyperglycemia leads to micro-vascular changes in the blood vessels, as well as basement membrane alteration in the salivary glands. This leads to increased leakage of glucose from the

ductal cells of the salivary gland, thereby increasing the glucose content in saliva [15].

This metabolic disease is a potential burden on both patients and society because of the high morbidity and mortality associated with infections and its renal, retinal and vascular complications. Thus, it is essential to assess the magnitude of the problem and take steps for the early detection and control of DM with regular monitoring over glycemic control [16]. There are so many studies conducted. Not only glucose level there is also alteration of whole salivary constituents, such as salivary sodium, potassium, proteins, amylase, albumin and IgA, and a possible explanation was sought to the prevalence and severity of periodontal disease, dental caries in diabetic mellitus and role of saliva that brought about these changes [17, 18]. The routinely employed investigative procedures for glucose monitoring are invasive, but saliva can best serve as a valuable non-invasive diagnostic aid. Thus, the correlation between salivary and plasma glucose levels would be helpful in monitoring diabetes noninvasively.

CONCLUSION

There are definite changes in salivary composition, with increased levels of salivary glucose, total protein and enzymes in diabetic and non diabetic patients. The present study shows a significant positive correlation between the plasma and salivary glucose levels in the cases and study population. The levels of glucose in plasma of diabetic patients could be reflected in saliva; hence the salivary glucose could be helpful for routine evaluation of diabetic patients. Detailed information on the possible influences of the salivary glucose levels and further studies which include random and postprandial glucose correlation would perhaps establish more definitively whether salivary glucose estimation would replace plasma glucose estimation one day. Such an event would be in the interest of the patient, since collection of salivary samples is an easy, safe and non-invasive procedure.

Introducere. Diabetul zaharat este o patologie gravă cu incidența în creștere ce încarcă sistemele de sănătate din toată lumea. În momentul de față diagnosticul diabetului se realizează analizând nivelul plasmatic al glucozei. Saliva oferă însă mai multe avantaje. Aceasta poate fi colectată non-invaziv chiar de către pacient. Studiul de față a avut ca scop estimarea și corelarea valorilor salivare ale glucozei comparate cu cele plasmatice.

Metode. Studiul a avut trei grupuri: grupul 1 cu pacienți cu diabet zaharat cu glicemie >200 mg/dL, grupul 2 cu pacienți diabetici ce au avut glicemii între 130-200 mg/dL și grupul 3 cu pacienți nediabetici (glicemie <130 mg/dL). Pacienților le-a fost determinată glicemia și le-au fost recoltate probe salivare pentru analiza glucozei salivare.

Rezultate. Nivelurile salivare au fost semnificativ statistic mai mari la grupurile 1 și 2 comparativ cu grupul 3. Glucoza salivară s-a corelat cu glicemia, sugerând că glucoza salivară ar putea fi utilizată drept instrument pentru estimarea glicemiei la diabetici.

Concluzii. Studiul a relevat că evaluarea glucozei salivare poate fi folosită drept un instrument noninvaziv și nedureros pentru evaluarea pacientului diabetic.

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