SALIVA - AN EFFICIENT TOOL IN THE ESTIMATION OF GLUCOSE LEVEL - A COMPARATIVE STUDY

ABSTRACT:

Diabetes mellitus requires a frequent monitoring of sera glucose levels in the body. The commonly used diagnostic fluid for detection of glucose levels is blood, but it is an invasive and painful procedure.

Methods: Twenty diabetic and non-diabetic subjects were randomly selected. The quantitative estimation of blood and salivary glucose levels was performed by glucose oxidase enzyme method using glucose oxidase-peroxidase kit.

Result: A correlation was observed between HbA1c and salivary glucose of diabetic as well as non-diabetic subjects. The result showed a highly significant positive correlation between salivary glucose and serum glucose in both diabetic patients and in controls.

Conclusion: From this study, it was concluded that salivary glucose level can be used as a noninvasive diagnostic, as well as a monitoring tool to assess the glycemic status of Type II diabetes mellitus patients.

Keywords: Saliva, Salivary glucose, HbA1c, Hyperglycemia, Diabetes mellitus

INTRODUCTION:

Diabetes mellitus is chronic diseases characterized by insulin deficiency, cellular resistance to insulin action, or both, resulting in hyperglycemia and other related metabolic disturbances [2]. Owing to lack of sufficient diagnosis and treatment, diabetes is a major cause of death worldwide, more than half of the diabetics remain undiagnosed especially the patients with Type 2 diabetes [1]. In glycated hemoglobin, where glycation occurs in hemoglobin by the nonenzymatic reaction between the glucose and the N-terminal end of the β-chain, which forms a Schiff base. During the rearrangement, the Schiff base is converted into Amadori products, of which the best known is HbA1c. In the primary step of glycated hemoglobin formation, the protein reacts with blood glucose to form aldimine in an exceedingly reversible reaction. In the

secondary step, which is irreversible, aldimine is gradually converted into the stable ketoamine form. The HbA present, are classified into a major fraction as HbA1, which in turn is made up of HbA1a1, HbA1a2, HbA1b, and HbA1c fractions, defined by their electrophoretic and chromatographic properties. HbA1c is the plethoric of these fractions and in health contains approximately 5% of the total HbA fraction. As mentioned above, glucose in the open chain format binds to the N-terminal to form an aldimine before undergoing an Amadori rearrangement to form a more stable ketoamine. This is a nonenzymatic process that occurs continuously *in vivo*. The formation of the glycated hemoglobin is a normal part of the physiologic function cycle. However, as the average plasma glucose increases, so does the amount of glycated hemoglobin in the plasma. This specific characteristic of the hemoglobin biomarker is utilized for estimating the average blood glucose levels over the previous two to three months [9].

Monitoring of glycated hemoglobin (HbA1c) levels has been an accurate measure of average glycemic control over the past three months [2]. Saliva is the principal defensive factor in the mouth which contains informative components that can be used as diagnostic markers for many human diseases [5]. Like serum, saliva is a complex biological adjunct containing a variety of hormones, antibodies, enzymes, anti-microbial, and growth factors. Many of those enter spittle from the humor by passing through the areas between the cells by transcellular (passive living thing diffusion and/or active transport) or para cellular (extra cellular ultra-filtration) routes. Therefore, most of the components found in the serum are also present in saliva, thus, making saliva functionally equivalent to serum in reflecting the physiological status of the body, including the hormonal, nutritional, and various metabolic variations [3]. Many studies have been proposed to demonstrate the raised salivary glucose level in diabetes [5-14]. However, very few studies are conducted comparing the glycated hemoglobin with the salivary glucose levels.

So this study aims to analyse if the salivary glucose levels can be used as a means of regular monitoring of DM without the need for invasive procedure required for sera glucose level estimations. The objectives in this study were 1) To calculate the Salivary Glucose Level in Diabetic patients. 2) To correlate the serum Glycated Haemoglobin level with Salivary glucose level. 3) To compare the Salivary glucose level with control and diabetic patients.

MATERIALS AND METHODS:

This study was conducted in the Department of Oral Medicine and Radiology, Best Dental Science college and Hospital, Madurai. The study was approved by the Institutional Ethical Committee. The samples were obtained from individuals who volunteered to participate in this study. The study has 2 groups of patients. 20 subjects with type 2 diabetes were classified as Study/Diabetic group and 20 healthy nondiabetic subjects with no systemic disorder were classified as Control group.

The inclusion criteria were,

- As per the current specifications (2016) of the American Diabetic Association (ADA) for diagnosis and monitoring control of the disease process in DM patients

The exclusion criteria were,

- Patients with other systemic illnesses/diseases
- Pregnant females
- Smokers and alcoholics
- Persons who were treated with radiotherapy in the head and neck region
- Patients on drugs supposed to have an impact on the glycemic status of the patients

The details and the need for the study were explained to the subjects and informed consent obtained. A detailed case history was taken followed by a general and oral examination. Salivary sample collection was performed in the morning hours between 9.00 a.m. and 11.00 a.m. immediately after obtainment of the sera samples.

Using a 2 ml sterile, disposable plastic syringe and a 24-gauge needle, the antecubital vein was punctured and 2 ml of whole blood was drawn. Serum was collected into Ethylene Diamine Tetra Acetic acid (EDTA) containing tube. The sample was centrifuged at 3000 rpm for about 5 min. One milliliter of glucose reagent was added to 10 µl of test sample and glucose standard. Both were incubated at 37°C for about 10 min. The absorbance values were measured on Erba Chem 7, semi-automated analyzer.

Passive collection of unstimulated whole saliva was done using Spit technique for the salivary samples. Salivary sample collection was performed in the morning between 9.00 a.m. and 11.00

a.m. immediately after obtainment of the sera samples. Patients were asked not to eat, drink, or smoke 2 h before salivary collection. The patients were asked to sit in the dental chair with head tilted forward and instructed not to speak, swallow, or do any head movements during collection of the sample. The patients were then instructed to spit the saliva into a sterile Eppendorf vial every minute for about 5 min. Saliva of about 2 ml was collected. The sample was centrifuged at 3000 rpm for about 20 min and clear supernatants were processed immediately for estimation of salivary levels glucose, amylase, and total protein. The test sample (100 μl) was mixed with the glucose reagent in a ratio of 1:3 and glucose standard and incubated at 37°C for 5 min. The absorbance values were measured on Erba Chem 7, semi-automated analyzer.

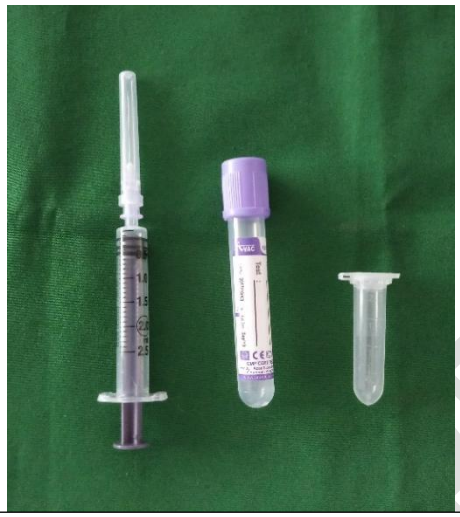


Fig 1: A sterile 2ml syringe, 3 ml EDTA infused tube for serum collection, 2.5 ml Eppendorf vial for saliva collection.

Fig 2: Semi automated analyzer

STATISTICAL ANALYSIS:

The data collected were compiled using MS-Office Excel and was subjected to Statistical analysis using IBM corp. SPSS (Statistical package for social sciences) Statistics for windows, version 20.0 (Armonk, NY) Statistical significance was set at $P < 0.05$. Descriptive statistics were used to analyze the data. Normality of the data was assessed. Chi-square test was done to assess the association between scores and measurements. Pearson correlation was done to assess the correlation between scores and measurements.

RESULTS:

The mean age of patients in the study group was 54.05 and that in the control group was 56.32. The mean serum glucose in control group was 4.80% with an SD of 0.46. In the diabetic group, the mean serum glucose level was 7.14% and SD was 0.64. Comparisons of blood glucose levels between the control and diabetic groups revealed that the difference was significant (P = 0.033) [Table 1]. The mean salivary glucose in control group was 5.26 mg/dL with an SD of 1.54. In the diabetic group, the mean salivary glucose level was 15.64 mg/dL and SD was 3.45 [Table 2]. Comparisons of salivary glucose levels between the control and diabetic groups showed that the difference was significant (P = 0.024). The correlation coefficient between serum glucose and salivary glucose was calculated in both control patients, and diabetic patients and the r value was found to be 0.84 and 0.074, respectively, which were statistically significant [Table 3]

Table 1: Comparison of serum glucose levels in Controls and Diabetics patients

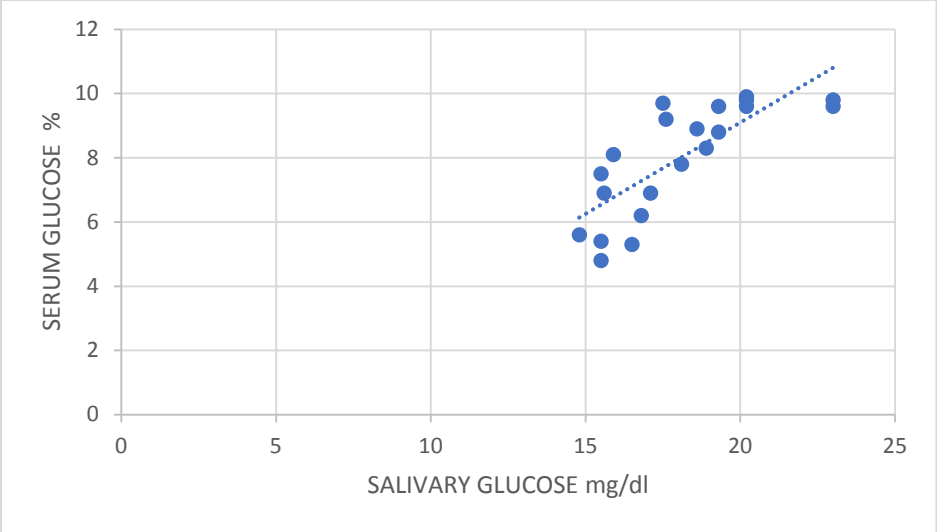
Group	Mean	SD	pValue
Control	4.80	0.46	0.033
Diabetics	7.14	0.64	

Table 2: Comparison of salivary glucose levels in Controls and Diabetics patients

Group	Mean	SD	pValue
Control	5.26	1.54	0.024
Diabetics	15.64	3.45	

Table 3: Correlation among serum and salivary glucose level in Control and Diabetic group

Variables	Salivary glucose level in controls	Salivary glucose level in diabetics
Serum glucose level in controls	r = 0.84	
Serum glucose level in diabetics		r = 0.074



Graph 1: Correlation between salivary and blood glucose levels in the diabetic group

DISCUSSION:

In our study, we found that salivary glucose values were higher among diabetics than in the controls; the difference was statistically highly significant ($P<0.05$). Arati et al. estimated salivary glucose using glucose oxidase method for eighty diabetic patients in their study. The results showed a significant positive correlation between salivary glucose levels and fasting blood glucose levels [1]. Kartheeki et al. similarly concluded that there exists a statistically significant correlation between serum and salivary glucose level on studying the diabetic patients [3]. Fleckseder and Carlson and Ryan reported the presence of sugar in the saliva of diabetic patients and other authors have reported increases in salivary glucose levels in diabetes mellitus patients in comparison to nondiabetics [15]. Similar to our study, Abikshyeet et al. obtained highly significant positive correlation between the serum and salivary glucose level, where diabetic and control patients were assessed [2]. However, Forbat et al. concluded that salivary glucose levels did not reflect blood glucose levels [16]. Similarly, Carda et al concluded that the salivary glucose levels of 76.4% of diabetic patients were in the normal range [17].

In this study, there was a positive correlation between salivary and serum glucose in diabetic patients, as well as the controls. These correlations were found to be statistically significant. Hence, it can be stated that salivary glucose can be used as an indicator of serum glucose

concentration in diabetic patients. The results of our study were in accordance with the study conducted by Abikshyeet et al.,[2] Amer et al.,[12] Agrawal et al.,[7] and Panchbhai et al [1].

Diabetes mellitus is characterized by the presence of hyperglycemia because of defective beta cells of pancreas secretion, its action or both. The chronic increased level of glucose in diabetes is related to long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease. Glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications [14].

Diabetes is often associated with increased basement membrane permeability, which can be attributed to the increased passage of molecules from exocrine glands into their secretions leading to an enhanced leakage of serum derived components into whole saliva via gingival crevices [11]. Glucose, a small molecule can easily diffuse through semipermeable membranes thus increasing the salivary glucose levels, which ultimately results in consequent loss of homeostasis and greater susceptibility to diseases in the oral cavity [4]. Amer *et al*. suggested that salivary samples of the nondiabetic control subject did not show the presence of glucose even in the slightest concentrations while the samples obtained from the type 2 diabetics (non IDDM) showed significant concentration of glucose in the saliva [3].

Darwazeh *et al*. conducted a study, wherein salivary glucose levels were analyzed by modified enzymatic ultraviolet detection method and found glucose concentration in saliva of diabetics to be significantly higher than in the controls and directly related to the sera glucose levels [12]. Belazi *et al*. conducted a study to examine the flow rate and composition of unstimulated whole saliva and serum in children with newly diagnosed insulin-dependent DM (IDDM) and compared the values derived with the values obtained for a group of healthy controls although they observed no significant difference in the salivary flow rates between the two groups while significantly higher concentrations of glucose in the saliva and serum in children with IDDM[12]. Vasconcelos *et al*. conducted a study to evaluate the correlation between sera and salivary glucose levels, wherein the saliva was stored frozen until use in the glucose assay while the absorbance values of salivary glucose assay were read on a spectrophotometer at wavelength of 500 nm [3].

Salivary glucose concentration was found to be significantly higher in type 2 diabetics although they could not observe a significant positive correlation between salivary and sera glucose levels in diabetic patients which was in contrast to the results of the present study. Jurysta *et al*. conducted a study to evaluate salivary glucose concentration in unstimulated and mechanically stimulated salivary samples in the normal, healthy controls and diabetic patients and observed higher glucose concentration in the saliva of diabetic patients than in the controls [3]. Sera glucose levels were measured by glucose oxidase method while salivary glucose levels were assessed by hexokinase method in their study. Furthermore, they found no significant difference between unstimulated and stimulated salivary samples when compared with the sera glucose levels in the diabetic patients.

CONCLUSION:

Seeing the present prevalence of DM on such a large scale globally, the analysis of saliva can offer a reliable, noninvasive and cost-effective approach for the screening of large populations, thereby, preventing the morbidity and mortality associated with this dreadful and complex metabolic disorder which seems to be attacking people in all age groups, genders and with varied socioeconomic status. Salivary estimation of glucose aids in diagnosing and for regular monitoring of glucose level highly significant compared with the serum-based methods. Nevertheless, further studies on larger populations and in different geographic areas are needed to establish salivary glucose estimation as a diagnostic as well as a monitoring tool for diabetes mellitus.

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