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

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ABSTRACT:

Diabetes mellitus necessitate a repeated observation of serum glucose levels in the body. The commonly used diagnostic fluid for finding the glucose levels in the blood, but it is an invasive and unpleasant custom.

Methods: Twenty diabetic and non-diabetic subjects were randomly selected. The perceptible assessment of blood and salivary glucose levels were performed by the 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 convinced positive correlation between salivary glucose and serum glucose in both diabetic patients and controls.

Conclusion: From this study, it was achieved that salivary glucose level was a nearly new noninvasive indicative technique, and used as a survey tool to estimate the glycemic state of Type II diabetes mellitus patients.

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

INTRODUCTION:

Diabetes mellitus is sustained condition indicating insulin insufficiency, cellular impedance to insulin response, or both, producing hyperglycemia and else analogous metabolic disturbances [2]. Due to shortage of ample diagnosis and treatment, diabetes is an extensive purpose of mortality globally, further half of the diabetics persist undiagnosed notably the inmates with Type 2 diabetes [1]. In glycated hemoglobin, glycation occurs in the hemoglobin by the nonenzymatic reaction amidst the glucose and the N-terminal end of the β-chain, that scheme a Schiff base. During the rearrangement, the Schiff base is transformed into Amadori output, whichever the leading familiar is HbA1c. In the fundamental stride of glycated hemoglobin arrangement, the protein reacts with blood glucose to form aldimine in an exceedingly varying return. In the collateral step which is permanent, aldimine is continuously transformed into the constant ketoamine form. The HbA was distributed into a large division as HbA1, that orderly includes HbA1a1, HbA1a2, HbA1b, and HbA1c section, described by their electrophoretic and chromatographic properties. HbA1c is enormous of these portions and in healthiness consists relatively 5% of the total HbA fraction. This is a nonenzymatic method that arises regularly *in vivo*. The development of the glycated hemoglobin is a regular portion of the physiologic function cycle. The average plasma glucose increases with a load of glycated hemoglobin in the plasma. This peculiar feature of the hemoglobin biomarker is promoted for measuring the mean blood glucose levels over the past two to three months [9].

Monitoring of glycated hemoglobin (HbA1c) levels has been a detailed part of mean glycemic control over the past three months [2]. Saliva is the chief averting aspect in the oral cavity that has descriptive components that can be used as diagnostic markers for the most humans' diseases [5]. Like the serum, saliva is an organized marker 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 paracellular (extracellular ultra-filtration) pathways. 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 of them are conducted comparing the glycated hemoglobin with the salivary glucose levels.

So this study aims to analyze if the salivary glucose levels can be used as a way of standard oversee of DM while not the requirement for the invasive procedure needed for sera glucose level estimations. The objectives 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 between control and diabetic patients.

MATERIALS AND METHODS:

This study was organized in the Department of Oral Medicine and Radiology, Best Dental Science College and Hospital, Madurai. The study was accepted by the Institutional Ethical Committee. The specimens were achieved from persons who proffered to take part in this study. This study has 2 groups of patients. 20 subjects with type 2 diabetes were divided as Study/Diabetic group and 20 healthful nondiabetic persons with no systemic disorder was 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 different systemic illnesses/diseases
- Pregnant females
- Smokers and alcoholics
- Persons who were managed with radiotherapy in the head and neck region
- Patients on medications supposed to have a strike on the glycemic status of the patients

The Specifics and the want for the study was explained to the participants and informed approval attained. A clear case history was taken pursued by a general and oral examination. Salivary specimen collection was taken in the morning hours between 9.00 a.m. and 11.00 a.m. instantly after the acquirement of the sera samples.

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

Passive collection of unstimulated whole saliva was done using Spit technique for the salivary specimens. Salivary sample collection was done during the morning session between 9.00 a.m. and 11.00 a.m. instantly after attaining the sera samples. Patients were requested not to eat, drink, or smoke 2 hours before salivary collection. The patients were asked to sit in the dental chair with head bend forward and asked not to speak, swallow, or do any head movements during the collection of the sample. The patients were then asked to spit the saliva into a sterile Eppendorf vial every minute for about 5 min. The saliva of about 2 ml was obtained. The sample was centrifuged at 3000 rpm for about 20 minutes and clear supernatants were processed immediately for estimation of salivary levels glucose. The test sample (100 µl) was assorted with the glucose reagent in a ratio of 1:3 and glucose standard and incubated at 37°C for 5 minutes. 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 exposed to Statistical analysis using IBM corp. SPSS (Statistical package for social sciences) Statistics for windows, version 20.0 (Armonk, NY) Statistical significance was established at $P < 0.05$. Descriptive statistics were used to analyze the data. Normality of the data was assessed. Chi-square test was done to determine the relations between scores and measurements. Pearson correlation was done to assess the correlation between scores and measurements.

RESULTS:

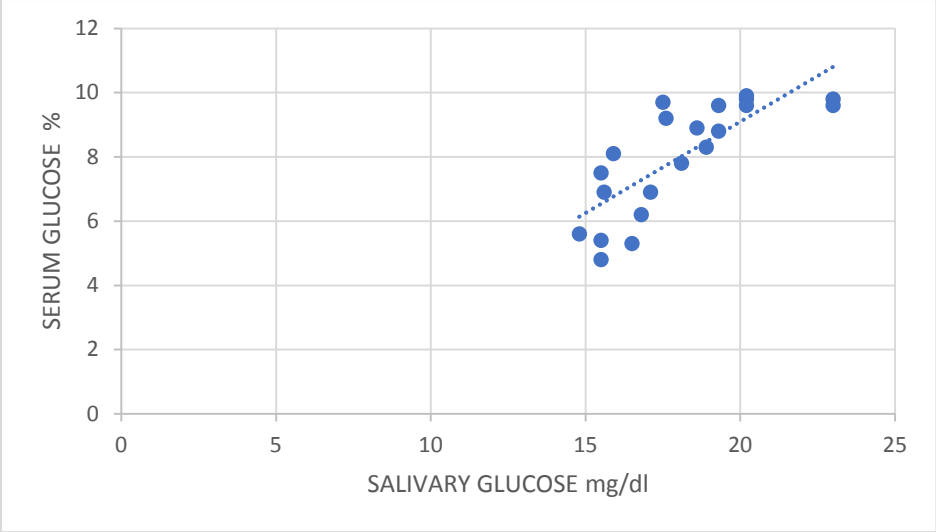
The mean age of patients in the study group was 54.05 and that in the control group was 56.32. The mean serum glucose in control group was 4.80 mg/dL with an SD of 0.46. In the diabetic group, the mean serum glucose level was 7.14 mg/dL and SD was 0.64. Comparisons of blood glucose levels among the control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

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	

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	

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 this study, we established that salivary glucose values were increased among diabetics than in the controls; the discrepancy 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 definite correlation between salivary glucose levels and fasting blood glucose levels [1]. Kartheeki et al. similarly concluded that there exists a statistically significant correlation among serum and salivary glucose level on studying the diabetic patients [3]. Fleckseder and Carlson and Ryan described the existence of glucose in the saliva of diabetic patients and other authors have expressed escalation in salivary glucose levels in diabetes mellitus patients in contrast to nondiabetics [15]. Analogous to our study, Abikshyeet et al.

obtained highly significant positive correlation amidst the serum and salivary glucose level, where diabetic and control patients were assessed [2]. However, Forbat et al. stated that salivary glucose levels did not follow blood glucose levels [16]. Furthermore, Carda et al declared that the salivary glucose levels of 76.4% of diabetic patients were in the normal range [17].

Here, there was a positive correlation between salivary and serum glucose in diabetic patients, as well as in the controls. These correlations were established 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 indicated by the existence 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%, places individuals at high risk of developing diabetes and its complications [14].

Diabetes is usually related to increased basement membrane permeability, which might be attributed to the multiplied passage of molecules from exocrine glands into their secretions resulting in an enhanced leakage of serum derived components into whole saliva via gingival crevices [11]. Glucose, a small molecule will simply diffuse through semipermeable membranes therefore increasing the salivary glucose levels, that ultimately ends in resultant loss of homeostasis and greater susceptibility to diseases in the oral cavity [4]. Amer *et al*. recommended that salivary samples of the nondiabetic control subject did not show the presence of glucose even in the slightest concentrations whereas the samples obtained from the type 2 diabetes (noninsulin-dependent diabetes mellitus) showed vital concentration of glucose in the saliva [3].

Darwazeh *et al*. conducted a study, whereby salivary glucose levels were analyzed by a changed enzymatic ultraviolet detection method and located glucose concentration in saliva of diabetics to be considerably over within the controls and directly associated with the sera glucose levels [12]. Belazi *et al*. conducted a study to look at 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 determined no vital difference in the salivary flow rates between the two groups whereas

considerably higher concentrations of glucose in the saliva and serum in children with IDDM[12]. Vasconcelos *et al*. carried out a study to judge the correlation between sera and salivary glucose levels, wherein the saliva was stored frozen till 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 considerably higher in type 2 diabetics though they may not find a major direct correlation between salivary and sera glucose levels in diabetic patients, that was in distinction to the results of the current research. In another study, salivary glucose concentration was evaluated in unstimulated and mechanically stimulated salivary samples in the normal, healthy controls and diabetic patients and higher glucose concentration was detected 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. Furthermore, no significant difference was found between unstimulated and stimulated salivary samples when compared with the sera glucose levels in the diabetic patients.

CONCLUSION:

Seeing the current prevalence of DM on such an outsized scale globally, the analysis of saliva can give a reliable, noninvasive and price-effective approach for the screening of enormous populations, thereby, preventing the morbidity and mortality related to this dreadful and complicated disorder that appears to be attacking people in all age groups, genders and with varied socioeconomic status. Salivary estimation of glucose aids in diagnosis and for normal monitoring of glucose level, that is highly significant compared with the serum-based strategies. Nevertheless, more studies on larger populations and in several geographic areas are required to determine salivary glucose estimation as a diagnostic additionally as a monitoring tool for diabetes mellitus.

CONFLICTS OF INTEREST:

There are no conflicts of interest.

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Nil.

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