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SALIVARY GLUCOSE LEVEL IN DIABETIC PATIENTS: A COMPARATIVE CROSS SECTIONAL STUDY

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ABSTRACT

Diabetes Mellitus is a chronic endocrine disease characterized by hyperglycemic state owing to insulin deficiency or its resistance. Its diagnosis and monitoring requires regular measurement of blood glucose level, which is very apprehensive and painful to the patients. Therefore it necessitates the need of less invasive body fluid like saliva, whose collection is easy, economical, and painless and doesn't require expertise. Hence this study was carried out to correlate the fasting salivary glucose with fasting serum glucose and glycated hemoglobin in diabetes patients and compare it with healthy control and to substantiate the role of saliva as a diagnostic tool. This was a hospital based comparative cross sectional study. We included 50 newly diagnosed case of diabetes and 50 healthy age and sex matched control after taking their informed consent. Five ml fasting unstimulated saliva and 5 ml fasting blood was collected under standard conditions and the sample were processed immediately. Salivary and serum glucose was estimated by hexokinase method in autoanalyzer. Glycated hemoglobin (HbA1c) was analyzed by turbidimetric inhibition immunoassay in autoanalyzer .The data were analyzed by Student's t-test and Pearson's correlation using SPSS version 16. The salivary glucose level was significantly higher in diabetic patients as compare to control ($p<0.001$). We also noticed a significant positive correlation between fasting salivary glucose and fasting serum glucose, ($r=0.762$, $p<0.001$) whereas correlation between fasting salivary glucose and glycated hemoglobin in diabetic patients was not significant($r=0.391$, $p=0.121$).

Our finding suggests that the salivary glucose is directly correlated with serum glucose and the cutoff value set by Receiver Operating Characteristics (ROC) curve is ≥2.55 mg/dl for diabetic patients.

Keywords: Diabetes mellitus, fasting salivary glucose, fasting serum glucose.

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder characterized by insulin deficiency, insulin resistance or both, resulting in hyperglycemic state which when left untreated can leads to serious renal, ocular, neural, vascular and plethora of complications. This readily impairs the quality of life and reduces the life span of the patients.[1,2] In United States alone 14 million people are affected and one third of them are undiagnosed.[3] The total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million in 2030.[4] Currently it is diagnosed and monitored by measuring blood glucose level and all the diagnostic kits available uses blood as sample. The regular pricking for monitoring the blood glucose becomes quite cumbersome and painful to the patients and this reduces the compliance. Hence this necessitates the need of other bodily fluid which is noninvasive to monitor the glucose level. In this regard saliva offers a distinctive advantages as it is non-invasive, easy to collect, expertise not required, cost effective and most importantly painless. In the recent years interest has been increasing in non-invasive diagnostic testing. All steroids of diagnostic significance in routine clinical endocrinology can now be measured readily in saliva.[5] Therefore the present study was undertaken to estimate and correlate salivary glucose with that of blood glucose and glycated hemoglobin. We also tried to compare the salivary glucose level between diabetes patients

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and healthy controls. It also aimed to find out the cutoff value for salivary glucose that can serve as the diagnostic range in diabetic patients.

MATERIALS AND METHODS

Study design

This comparative cross sectional study was carried out in the Biochemistry Division of Central Laboratory Services, B.P. Koirala Institute of Health Sciences, Dharan from March-July 2014. Fifty freshly diagnosed diabetes patients and same number of healthy age and sex matched controls were enrolled in the study only after taking their informed consent. A detail history from the patients and control was taken in the proforma.

Sample collection and processing

Fasting blood and saliva collections were carried out in the morning. Detailed information about the collection procedure of saliva was given to the participants. They 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 5 minutes into the sterile sample collection container. Five ml of venous blood was drawn immediately after saliva sampling of which 3 ml was transferred in Sodium Flouride (NaF) vial and 2 ml in Ethylenediaminetetraacetic acid (EDTA) containing vial. Upon completing sample collection, the specimens were centrifuged at 3800 g for 10 minutes, and then the serum and saliva supernatants were isolated and stored at -20°C for later analysis of glucose and glycated hemoglobin.

Biochemical analysis

Salivary and serum glucose was analyzed by enzymatic hexokinase method in autoanalyzer (cobas c III, Germany). Glycated hemoglobin (HbA1c) was analyzed by turbidimetric inhibition immunoassay in autoanalyzer (cobas c III, Germany).

Statistical analysis

The data were presented as mean ± SD using Microsoft office Excel 2010. These were then compared using Pearson's correlation coefficient and independent Student's t-test. A p value <0.05 was accepted as significant and a value <0.001 was considered highly significant. ROC curve was also plotted to find out the sensitivity and specificity of the assay and assess its diagnostic ability to use fasting salivary glucose for prediction of diabetes. Analyses were performed using SPSS software version 16.

RESULTS

In this study the age ranged from 18 to 86 years in diabetes patients and from 23 to 79 years in the control group. We also found that the diabetes patients were overweight as compare to control group and is depicted in Table 1. Comparison of fasting salivary glucose and fasting serum glucose in diabetic patients and control group has been made and is depicted in Table 2.

Table 1: General characteristics of the participants

	Diabetics	Control
Age in years	51±14.9	49±7.1
Sex		
Male (%)	13(26)	16(32)
Female (%)	37(74)	34(68)
BMI (Kg/m^2)	26.38±3.27*	24.27±2.70

*p<0.01 statistically significant as compared to control group. Data expressed as mean±SD and frequency.

Table 2: Comparison of fasting salivary and fasting serum glucose level in diabetics and controls

Parameters	Diabetics	Controls
Fasting salivary glucose	3.0±1.0*	1.3±0.6
Fasting serum glucose	158±64*	80±11

*p<0.001 statistically significant as compared to control group. Data expressed as mean±SD.

There was positive correlation between fasting salivary glucose and fasting serum glucose in diabetes patients (r=0.762, p<0.001, Figure 1). The correlation coefficient between serum glucose and salivary glucose in control group was calculated and the r value was found to be 0.631, which was highly significant (p<0.001, Figure 2). It is worth noting that the significance of the study group was much greater than that of the control group. The HbA_1c level ranged from 3.1% to 14.7%, with a mean of 7.17% and SD of 2.95. The correlation coefficient between HbA_1c level and fasting salivary glucose in diabetes

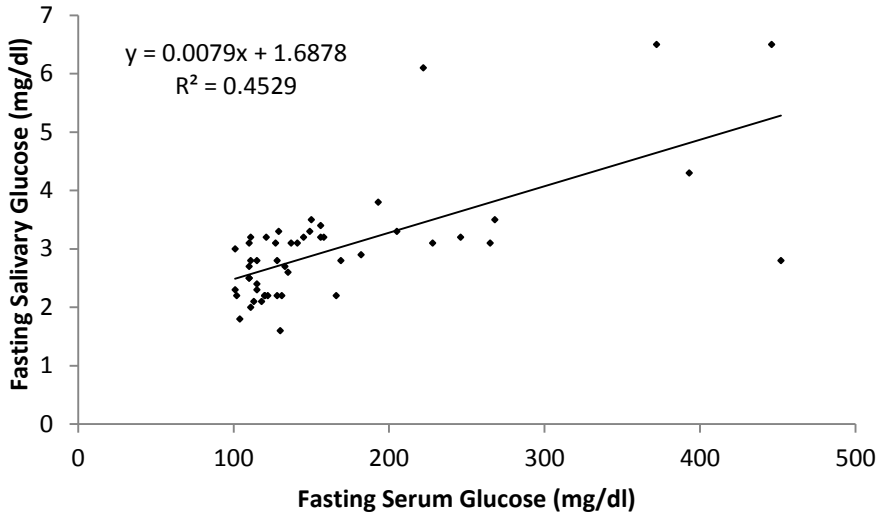

Figure 1: Correlation between fasting salivary and fasting serum glucose level in Diabetic Patients

patient was calculated and the r value was found to be 0.391, which was not significant (p =0.121) as shown in Figure 3.

From the ROC curve as shown in Figure 4, area under curve of fasting salivary glucose for the diagnosis of diabetes was calculated as 0.934.The cut off value of fasting salivary glucose for diagnosis of diabetes was found to be >2.55mg/dl at which sensitivity was 86.7% and specificity was 90%.

insulin action, or, most commonly, both. 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.[6]

For diagnosing and monitoring the glucose level in diabetes till date the blood is the choice of sample that is obtained by venipuncture technique which is traumatic. Hence the need for an alternative

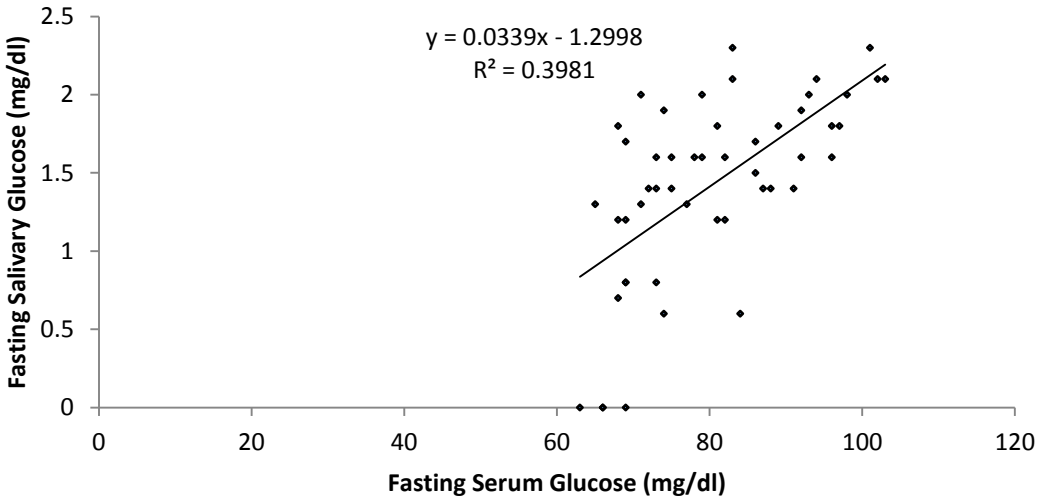

Figure 2: Correlation between fasting salivary and fasting serum glucose level in controls.

DISCUSSION

Diabetes mellitus is a group of metabolic disorders that is characterized by hyperglycemia. It may be because of defects in insulin secretion,

noninvasive technique arises. This has attracted many researchers and scientists to use the saliva as the choice of body fluid for diagnosing and monitoring various diseases.[7-12]

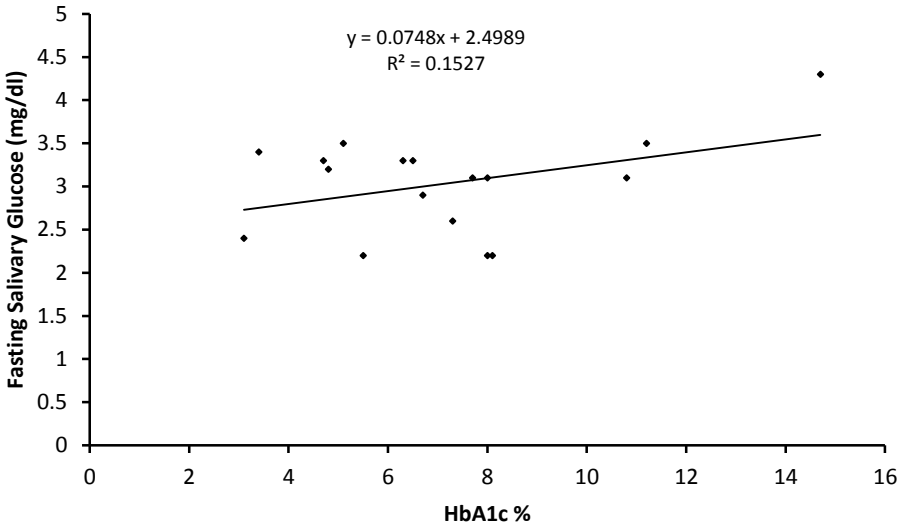

Figure 3: Correlation between fasting salivary glucose and glycated hemoglobin in Diabetic Patients

We all know that glucose is present in saliva but exact mechanism of its secretion into saliva is not clear. Though some mechanism has been proposed[13-15] but it is still a hypothesis and not an established fact. As glucose is a small molecule it easily diffuses through the semipermeable membrane. Thus, large amounts of glucose becomes available to the saliva when blood glucose levels are elevated as in diabetes. Other probable explanations given for the presence of glucose in saliva are diabetic membranopathy. Hyperglycemia leads to increased advanced glycosylation end products, commonly known as "AGEs". These AGEs crosslink proteins such as collagen and extracellular matrix proteins, leading to basement membrane alteration and, hence, endothelial dysfunction. This alters the microvasculature structure and makes it more permeable i.e. alterations in the permeability of basement membrane of salivary glands which leads to leakage of glucose in saliva.

In the present study we investigated the fasting salivary glucose level in diabetics and compared it with the controls and found that there was significant rise in salivary glucose level in diabetics ($p<0.001$). Similarly there was significant rise in serum glucose level in diabetics as compared to controls ($p<0.001$). Reporting of the presence of glucose in saliva goes way back in the early 20th century.[16] Our findings was in accordance with many similar studies done in the past.[17-23] However few studies contradicts to our findings. For instance Forbat et al concluded that salivary glucose levels did not reflect blood glucose levels. Similarly, Carda et al concluded that the salivary glucose levels of 76.4% of diabetic patients were in the normal range.[24-25]

In our study, there was a positive correlation between salivary and serum glucose in diabetic patients. A correlation between salivary and serum glucose was found in the controls as well. These correlations were found to be statistically significant. Hence, salivary glucose appears to be an indicator of serum glucose concentration in diabetic patients. Similar to our study, some studies[17-26] found a positive correlation between salivary glucose and serum glucose. However, in contrast to our study, few trials[27] could not establish a correlation between salivary and serum glucose.

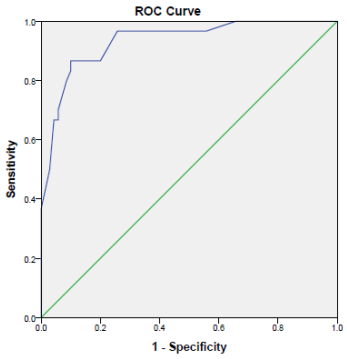

Figure 4: ROC curve for the hexokinase test used for fasting salivary glucose estimation in the participants.

We also estimated the HbA1c levels in diabetic patients and didn't find any significant correlation between HbA1c percentage and salivary glucose level. This is in accordance with the study of Lopez et al[13] who did not find any correlation between salivary glucose level and HbA1c percentage.

ROC curve was prepared to find out the sensitivity and specificity of the test used to estimate fasting salivary glucose level in the study participants. As per Figure 4 the fasting salivary glucose value of ≥2.55mg/dl is most sensitive and specific predictive value for diagnosis of DM.

Therefore, it can be concluded that fasting salivary glucose level can be used as a noninvasive diagnostic , as well as a monitoring tool to assess the glycemic status of diabetes mellitus patients. 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.

Conflict of interest

The authors declare no any conflict of interests for whatsoever.

Authors' contributions

All the authors confirmed their contribution in conceptualizing the study, collection of the samples, lab analysis, data collection, analysis and its interpretation. Manuscript writing, editing and review was also done by the authors.

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