**SALIVARY GLUCOSE AS A DIAGNOSTIC TOOL IN TYPE II DIABETES MELLITUS**

**A CASE-CONTROL STUDY**

**ABSTRACT:**

**Background and Objectives:** The prevalence of diabetes mellitus is increasing steadily in India. Understanding blood glucose level is the key to both diagnosis and management of diabetes mellitus. However, there is an on-going need for improvements in non-invasive, point-of-care tools for the diagnosis and prognosis of diabetes. Assessing a relationship between the blood glucose level and its concentration in other body fluids such as the saliva can help in developing a conservative method for blood sugar assessment replacing venous blood sampling. Diabetes mellitus is known to cause changes in salivary composition. Hence, this study was undertaken to evaluate the relationship of blood glucose level with salivary glucose in diabetic and non-diabetic patients.

**Materials and Method:** The study sample included 100 diabetic patients and 100 non-diabetic patients aged above 35years of age. Fasting blood and salivary glucose levels were measured in the two groups. Pearson’s correlation coefficient was used to assess the correlation of blood glucose with salivary glucose in the two groups.

**Results:** The results of the study revealed an increase in the level of fasting salivary glucose in diabetics compared to that of non-diabetic patients. It also showed a highly significant positive correlation between fasting salivary glucose and serum glucose in both diabetic patients and in controls.

**Conclusion:** From the present study, it can be concluded that fasting salivary glucose level can be used as a non-invasive diagnostic, as well as a monitoring tool to assess the glycemic status of type II diabetes mellitus patients.

**Key words:** Type IIDiabetes mellitus, fasting serum glucose, fasting salivary glucose

**INTRODUCTION:**

Diabetes mellitus is a clinically and genetically heterogenous metabolic disease characterized by abnormally elevated hyperglycemia and dysregulation of carbohydrate, protein and lipid metabolism. The primary feature of diabetes mellitus is chronic hyperglycemia, resulting from either a defect in insulin secretion from pancreas or resistance of body’s cells to insulin action or both.

Recent estimates indicate that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 20301.According to WHO estimate, 70% diabetics reside in developing countries, and India has world’s largest diabetes population with 50.8 million people suffering from diabetes followed by China2. In 2025, it is approximated that 57.2 million diabetics will be noticed in India3.

Type II diabetes mellitus is the fifth most common condition and the sixth leading cause of mortality amongst the elderly. India is world`s second most populous country with significant number of patients with type II diabetes2. Type II diabetes is associated with serious complications of the eyes, kidneys, heart, blood vessels and other organ systems. The quality of life is impaired with shortening of the patient’s lifespan4.

Currently, a diagnosis is achieved by evaluating blood glucose levels. The blood glucose level above 126mg/dl is diagnosed as diabetes. Monitoring blood glucose at frequent intervals causes unnecessary discomfort and mental trauma to patients and hence a much simpler and non-invasive technique for the diagnosis and monitoring of diabetes is very desirable.

Saliva is the principal defensive factor in the mouth which contains informative components that can be used as diagnostic markers for human disease. A growing number of dental and medical professionals are also finding that saliva provides an easily available, non-invasive diagnostic medium for a rapidly widening range of diseases and clinical situations. The multifarious components within saliva not only protect the integrity of the oral tissues, but also provide clues to local and systemic diseases and conditions.

The present study aimed to determine any alterations in the salivary glucose levels and to assess for a correlation between salivary glucose levels and blood glucose levels in both diabetic and non-diabetic patients. Salivary glucose was chosen as a medium to measure blood glucose because saliva is said to be the ultra-filtrate of blood. Glucose is one of the blood components that are transferable across the salivary gland epithelium in proportion to its concentration in blood.

**MATERIALS AND METHODS:**

The study group comprised of 200 patients aged above 35 years, who visited the out patient department of The Oxford Dental College and Hospital. Further the study group was divided as 100 diabetics and 100 non diabetic aged matched healthy control group. Patients taking treatment for other systemic diseases and pregnant women were excluded from the study group. Written consent was obtained from all the subjects taking part in the study and a data sheet was completed detailing the person’s name, age, sex, and relevant medical history. The subjects were asked to report to the clinic in the morning, on an empty stomach, after 8 hours of fasting. Before saliva collection, the patients were asked to rinse their mouth with distilled water and were asked to relax for 5minutes.The patients were asked to lean their head forward over the test tube and open their mouth slightly and saliva was allowed to drain into the tube for about 5mins.Any remaining saliva after 5mins was asked to spit into the test tube.

Following the collection of saliva sample, 5ml of venous blood was collected in a sterile test-tube. One millilitre of each unstimulated saliva and blood samples was centrifuged separately at 3000rpm for 20mins and clear supernatants was processed immediately for estimation of glucose.

**Procedure for salivary glucose estimation**

Salivary glucose was analysed by glucose oxidase end point assay using Abcam’s Glucose Assay Kit reagents. Three test tubes were taken.10µl of standard solution and 1.0ml of enzyme reagent was added into one test-tube and was marked as ‘S’. In another test-tube 10 µl of saliva sample was pipetted along with 1.0ml of enzyme reagent. In the third test-tube 10µl of serum sample was pipetted along with 1.0ml of enzyme reagent. The contents of all the three test-tubes were mixed well and incubated at 37°C for 5minutes.The absorbance of the samples were assessed using a colorimeter. The amount of absorbance is proportional to the level of glucose in the saliva and serum.

**STATISTICAL ANALYSIS:**

Data were analyzed using statistical software (SPSSversion 12 for Windows). Means and standard deviations (SDs) were calculated for the individual groups. Independent Student’s *t-*test ANOVA was used for testing between-groups variation, and Spearman’s coefficient was used for measuring the association between variables. A P-value < 0.05 was accepted as significant and a value < 0.01 was considered highly significant.

**RESULTS:**

The mean age of patients in the study group was 53.01 and that in the control group was 52.42. The mean serum glucose in diabetic group was 136.30mg/dL with a SD of 28.58. In the control group the mean serum glucose level was 97.78mg/dL and SD was 6.39.Comparisons of blood glucose levels between the control and diabetic groups revealed that the difference was highly significant(P=0.00001)(Table 1).The mean salivary glucose in diabetic group was 8.47mg/dL with a SD of 4.20. In the control group the mean salivary glucose level was 1.20mg/dL and SD was 0.86(Table 2).Comparisons of salivary glucose levels between the control and diabetic groups showed that the difference was highly significant (P=0.00001).

The patients were subdivided into three subgroups depending upon their serum glucose levels. The salivary glucose levels of the three subgroups were compared using one way ANOVA and the difference was found to be highly significant(P < 0.01).Further, to evaluate the difference between the groups a Tukey’s multiple posthoc procedure was performed. The results showed that each group had a statistically significant difference (P <0.01) in the salivary glucose levels.

The correlation coefficient between serum glucose and salivary glucose was calculated in both diabetic patients and healthy controls and the *r* value was found to be 0.8809 and 0.6342 respectively, which were statistically significant (*P* 0.05).

**DISCUSSION:**

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease5. National Urban Diabetes Survey (NUDS) study done in 2001 showed that Bengaluru had 12.4% prevalence of type II diabetes6.

Blood glucose monitoring by the patient and the physician is an important aspect in management of diabetes in order to control the debilitating complications of the disease. Conventional monitoring techniques require blood sample and the invasive procedures often cause pain and discomfort which may limit frequent testing.

With ever improving advances in diagnostic pathology, the race for the next generation of bloodless, painless and accurate glucose instruments has begun. So the other biological fluids like urine, sweat and saliva are also being utilized for the diagnosis of diabetes mellitus. Of these, saliva offers distinctive advantages because it can be collected non- invasively by individuals with modest training. Furthermore saliva may provide a cost effective approach for the screening of large populations.

The mean age of the study group was 53.01, which was higher than that reported by Prathiba et al8 and Panchbai et al9 where the mean age was 46.5 years and 48.5 years respectively. These data highlights the occurrence of type II diabetes mellitus in a relatively younger age group especially in developing countries as reported by CURES (Chennai Urban Rural Epidemiology Study)10.

In the current study the mean serum fasting glucose levels in diabetic group were 136.30 mg/dl with SD being 28.58. The mean serum glucose in controls was 97.78 with SD of 6.39.

In the present study salivary glucose concentration of diabetic and non-diabetic individuals was analysed. Among the diabetic patients mean salivary glucose levels in fasting state was 8.47mg/dL with SD of 4.20.In comparison, the control group had a lower mean salivary glucose of 1.20mg/dL with a SD of 0.86. Maximum salivary glucose in control group level did not exceed 5mg/dl which was much lesser than least values of diabetic patients. Similar to our study, S .O. Mahdavi et al.11, have reported the presence of sugar in the saliva of diabetic patients and many other authors including Panchbhai et.al 9, Sathya priya et.al12, Amer S et.al 13, Agrawal RP et.al14 , Panda et.al15 and Ivanovski K et.al16 have also reported increases in salivary glucose levels in diabetes mellitus patients in comparison to non-diabetics. However, contradictory to our study Bakianian Vaziri et al17 concluded that there was no statistically significant difference in salivary glucose concentrations between diabetic patients and control patients. Similarly, Carda et.al18 concluded that the salivary glucose levels of 76.4% of diabetic patients were in the normal range.

In the present 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 al15, Shehla Amer et al13, Agrawal et al 14 and Panchbhai9. Our study had a slightly higher positive correlation of about 0.8809 in diabetes patients and 0.6342 in controls compared to the other similar studies. However, in contrast to our study, the studies by Cedric Jurysta et al19, Carmen Carda et al18 and Ana Carolina et al 20 failed to establish a correlation between salivary glucose and serum glucose and they concluded that salivary glucose is not directly influenced by glycaemia and hence cannot be used to monitor glycemic control in diabetics. Again these contradictory results may be due to the difference in the selection criteria and the study method. Moreover, researchers from Brown University have developed a new biochip sensor that can selectively measure concentrations of glucose in human saliva and they concluded that it was an important step that would enable people with diabetes to test their glucose levels without drawing blood21.

In the current study, we divided the diabetic group into three subgroups based on their serum glucose levels. We compared the salivary glucose levels of these three subgroups. On comparing the subgroups, a statistically significant difference in salivary glucose was observed in the three groups. The study showed that patients with serum glucose levels below 130mg/dL had a mean salivary glucose of 5.43;patients with serum glucose levels between 130 and 200 mg/dL had a mean salivary glucose of 10.99mg/dL and patients with serum glucose level above 200mg/dL reflected a mean salivary glucose of 21.00mg/dL. From these data it can be noted that salivary glucose above 5.5mg/dL have a high chance of having serum glucose levels above 130mg/dL. Abikshyeet et al18 in their study concluded that salivary glucose levels above 4 mg/dL have a very high chance of having serum glucose levels above 130 mg/dL. Due to method variation and the population selected a slight variation is noticed from the current study.

In the present study a highly significant positive correlation was found between salivary glucose and serum glucose in both diabetic patients and in controls and a regression coefficient was calculated. The regression coefficient gives the amount of increase or decrease in the serum glucose for a unit change in the salivary glucose. Hence, from a given value of salivary glucose, we can predict serum glucose level by using the regression equation. The respective formulae are:

 Serum glucose =5.999(Salivary glucose) + 85.519 in Diabetic Group

 = 4.7254 (Salivary glucose) + 92.095 in Control Group

Since regression formulas derived for serum glucose shows to be population specific as the mean age and standard deviation varied according to the populations involved, we recommend further studies involving large samples in different populations.

From the current study it can be inferred that glucose can be detected in saliva of both diabetic and non-diabetic patients and the fact that the salivary glucose levels are higher in diabetics compared to that of healthy controls.The assessment of glucose levels to detect diabetes non-invasively will facilitate interventions designed to prevent or delay progression to uncontrolled diabetes and its complications.

**LIMITATIONS OF THE STUDY:**

A lot of factors influence the secretion of saliva and the constituents of saliva. Since raised glucose level is also recorded in GCF, the glucose in the saliva may not be exclusively of salivary gland origin. Hence the technique we adopted requires validation and future studies has to be concentrated on glucose estimation from specific glandular saliva.

**CONCLUSION:**

The present study highlights the possibility of using saliva as a diagnostic tool to assess the glucose concentration in blood, thereby making self-measurement of glucose less invasive. From our study it could be concluded that glucose was detectable in saliva of diabetic and non- diabetic individuals. A significant positive correlation was established between blood glucose and salivary glucose levels. From the current study an equation was formulated to assess the serum glucose level, utilizing the measured salivary glucose level. Hence, it can be concluded that fasting salivary glucose level can be used as a non-invasive diagnostic, as well as a monitoring tool to assess the glycaemic status of diabetes mellitus patients. This study highlights the role of dentists in diagnosing diabetes mellitus via salivary samples and hence promoting health for all in the society.

Further research should be focused on the correlation between salivary and serum glucose with a standardized technique. It is possible that tests based on saliva can have a substantial role in diagnosis and also early detection of diabetes mellitus. Also further research has to be done to know the standard range of salivary glucose levels for diagnosing diabetes mellitus.

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