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Research Article

SALIVARY GLUCOSE CONCENTRATION IN TYPE-II DIABETIC PEOPLE: A CASE–CONTROL STUDY

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ABSTRACT

Objective: The objective of the present study was to find a non-invasive method of sample collection that can be used to diagnose and monitor diabetes mellitus (DM).

Methods: In this study, saliva as a diagnostic fluid was collected noninvasively from subjects with modest training and this offers a cost-effective method for screening diabetes. To evaluate the association of blood glucose level with salivary glucose in Type-II diabetic mellitus (Type-II DM) pattients, a case–control study was conducted on 200 test and 200 healthy control people in selected study village in Kanchipuram (District). The glucose level was measured in saliva and blood plasma by glucose oxidase and peroxidase method.

Results: A highly significant positive correlation between fasting salivary glucose (69.377±14.329 mg/dl) and plasma glucose (249.935±64.65 mg/dl) in diabetic patients and in control group, plasma glucose level 117.545±10.595 and saliva glucose level 49.271±13.795 mg/dl was observed.

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

Keywords: Blood glucose level, Salivary glucose level, Diabetes mellitus, Saliva, Hyperglycemia.

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INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous (clinically and genetically) metabolic disease characterized by hyperglycemia and dysregulation of carbohydrate, protein, and lipid metabolism [1]. The main feature of DM is chronic hyperglycemia, resulting from either a defect in insulin secretion from pancreas or resistance of body's cells to produce insulin or both. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, and unexpected weight loss [2]. DM in India is gaining the status of a potential epidemic disease with more than 62 million people diagnosed with DM [3,4]. In the year 2000, India topped the world with the highest number of people having DM (31.7 million) followed by China (20.8 million) in the second place and the United States (17.7 million) in the third place [5-8].

Blood has been the most commonly used diagnostic fluid for the analysis of its various constituents. This study is focused on the fact that saliva being the principle and defensive fluid in the mouth with informative components can serve as diagnostic tool for human diseases. Collection of saliva is cost effective and non-invasive when compared to blood. The present study is proposed to measure the correlation between salivary and blood glucose levels in diabetic and non-diabetic subjects.

METHODS

Sample collection, processing, and storage

Diabetes camp was conducted to collect the sample from 200 control group and 200 Type-II diabetic people with different age groups from the selected study area of Thiruporur, Kanchipuram (District). The study was approved by the members of the Institutional Ethical Committee. Exclusion criteria for the control group were pregnancy, alcohol dependency, smoking, chronic diseases, and history of diabetes. After explaining and obtaining an informed consent, all subjects were advised to come on overnight fasting (12 h) before collection of unstimulated whole saliva samples. Denture wearers removed their dentures before saliva collection. Before collection, the subjects rinsed their mouth with water. After 5 min, the saliva was collected in the graduated collection test tube [9-14]. Flow rate was measured by recording the reading in the tube. The saliva samples were centrifuged at 4000 rpm for 15 min to remove any particulate material and then supernatant was transferred to a sterile vial immediately, stored at −20°C for further investigations.

Estimation of salivary glucose

Principle

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red-colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of glucose present in the sample.

$$\alpha - D - \text{Glucose} \xrightarrow{\text{Mutarotase}} \beta - D\ \text{Glucose}$$

$$\beta - D - \text{Glucose} + H_2O_2 \xrightarrow{\text{Glucose Oxidase}} D - \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + 4\ \text{Aminophenazone} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoeimine} + 4H_2O_2$$

Reagent

Ready to use reagents provided by the kit.

Sample

The sample was plasma and saliva.

Procedure

Wavelength/filter: 505 nm
Temperature: 37°C/RT
Light path: 1 cm
Pipette into clean dry test tubes labeled as blank (B), standard (S), and test (T).

Additional sequence	B (ml)	S (ml)	T (ml)
Distilled water	0.01	-	-
Glucose standard (S)	-	0.01	
Sample–plasma	-	-	-
Fasting/postprandial/saliva			0.01
Glucose reagent (GOD-POD)	1.0	1.0	1.0

GOD-POD: Glucose oxidase-peroxidase

Mix well and incubate at 37°C for 10 min. Measure the absorbance of the standard (Abs. S) and test (Abs. T) against the blank, within 60 min at 505 nm and record the readings.

Calculation

$$\text{Total glucose concentration in mg / dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

Statistical analysis

Statistical analysis was performed using GraphPad software. Descriptive statistics on each study variable including mean and standard deviations were analyzed. The results obtained from the analysis were tabulated expressed using mean±SD with their level of significance at p<0.0001.

RESULTS

The results were tabulated (Tables 1 and 2).

The observed results were given as mean±SD with their level of significant at p-value.

The fasting and postprandial mean plasma glucose concentration in the test group (155.48±45.577 and 249.935±64.65 mg/dl) were higher than the control subjects. The mean of fasting plasma glucose concentration was 92.495±8.053 mg/dl and postprandial plasma glucose concentration was 117.545±10.595 mg/dl in the control group (Table 1). Significantly elevated plasma glucose concentration was observed in diabetic patient than the control people at p<0.0001.

Salivary fasting glucose concentration of the test group (69.377±14.329 mg/dl) was significantly higher as compared with control subjects (49.271±13.795 mg/dl) (Table 2).

DISCUSSION

Monitoring the blood glucose by the physician is important for monitoring diabetic control. The routine diagnostic sample is usually blood being invasive, painful, causing discomfort, and may also limit frequent testing of blood sugar level in diabetic patients. The increased blood glucose readily diffuses through the semipermeable membrane and can be detected in saliva also. Salivary glucose assessment may provide a cost-effective approach as saliva is convenient diagnostic fluid for screening large population. In the present study, glucose concentration in blood and saliva of selected study population (control and diabetic people) was analyzed and compared.

Fasting blood sugar and postprandial blood sugar and fasting salivary glucose concentration were measured in control and diabetic people. The fasting and postprandial blood mean sugar level in diabetic patients are significantly higher (p<0.0001) than control subjects. A significantly elevated salivary glucose was observed in diabetic patients than the control subjects. The present study result was found to be similar with other scientific reports [15-22]. Kortuem was the first person to study correlation between blood glucose level and salivary glucose level in 1944 [23] followed by Shannon *et al.* [24]. Later on, Englander *et al.* [25] and Campbell, in 1965, shed light on it [26]. Carlson and Ryan (1908) reported the presence of sugar in saliva of diabetic patients [27] and other authors have also reported the increase in salivary glucose levels in diabetics over the non-diabetics [15-22].

Table 1: Comparison of controls and diabetics with blood glucose levels

Parameter	Blood		p-value
	Control (n-200)	Test (n-200)	
Fasting blood glucose (mg/dl plasma)	92.495±8.053	155.48±45.577	0.0001***
PP glucose (mg/dl)	117.545±10.595	249.935±64.65	0.0001***

n–Number of subject (control–200, test–200). Fasting blood levels were compared between control and test group, the values were statistically significant at p=0.000***, PP: Postprandial

Table 2: Comparison of Controls and Diabetics with salivary glucose levels

Parameter	Saliva		p-value
	Control (n-200)	Test (n-200)	
Fasting glucose (mg/dl)	49.271±13.795	69.377±14.329	0.0001***

n–Number of subject (control–200, test–200). Fasting salivary glucose levels were compared between the control and test group, the values were statistically significant at p=0.000***

Darwazeh *et al.* in their study correlated the salivary glucose concentrations with the plasma glucose concentration and indicated the presence of glucose in saliva of diabetics reflects the high plasma glucose concentrations [11]. However, Reuterving *et al.* (1987) reported that the salivary glucose concentration was low during better metabolic control period [15].

Similar to our study, Mahdavi *et al.* [28] have reported the presence of sugar in significant amount in saliva of diabetic patients and many other authors including Panchbhai *et al.* [29], Priya *et al*. [30], Amer *et al*. [19], Agrawal *et al*. [31], Abikshyeet *et al*. [32], and Ivanovski *et al*. [33] have also reported the increase in salivary glucose concentration in DM patients when compared to non-diabetics. However, contradictory to our study, Bakianian Vaziri *et al*. [34] concluded that there was no statistically significant difference in salivary glucose concentration between diabetic patients and control subjects. Vasconcelos *et al*. (2010) also reported a significantly higher salivary glucose level in diabetics when compared to non-diabetics irrespective of periodontal disease [35]. In this study, salivary glucose concentration is higher in diabetics when compared to that of healthy controls.

CONCLUSION

A significant positive correlation was established between blood glucose and salivary glucose levels. Hence, it can be concluded that fasting salivary glucose level can be used as a non-invasive diagnostic tool to monitor diabetes. It is possible that tests based on saliva can have a substantial role in diagnosis and also early detection of DM.

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AUTHORS' CONTRIBUTIONS

The first author has done the work and compiled the information. The second and third authors have reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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