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Original Article

Estimation of Salivary Glucose levels as a Diagnostic aid for Diabetes Mellitus

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ABSTRACT

Aim: The present study was designed to correlate the serum and salivary glucose levels and estimate associated salivary parameters (such as salivary flow rate, pH and buffering capacity) among diabetic and healthy individuals. **Settings and Design:** 80 patients were included in this study of which 30 patients were uncontrolled diabetics (group I), 30 patients were controlled diabetics (group II) and 20 patients were healthy individuals (group III). **Materials and Methods:** Salivary and serum glucose levels and salivary flow rate were measured using established methods. **Results:** The mean serum glucose was higher in group I as compared to group II and group III. In all the groups, salivary glucose levels significantly correlated with serum glucose levels. A statistically significant correlation was also observed between salivary glucose and salivary flow rate in all the three groups. The mean pH values were similar in all the three groups, indicating unaltered salivary buffering capacities. **Conclusion:** Salivary glucose levels may be used as an alternative and reliable index in diagnosis of diabetes mellitus.

Key words: Diabetes, Saliva, Diagnostic tool, Glycemia, Non-invasive diagnosis, patient care.

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INTRODUCTION

Diabetes is a major public health problem at present.[1] It represents a growing medical disorder, with concomitant morbidity and mortality affecting people of all ages. It is a syndrome of abnormal carbohydrate, fat and protein metabolism, which due to the absolute or relative lack of insulin results in acute and chronic complications.[2] In Asia, Indians seem to be at a greater risk of developing Diabetes. In urban areas, the crude prevalence rate of diabetes is about 9% and in rural areas it has increased to around 3% of the total population.[3] The prevalence of diabetes has increased 30 to 40 percent during the past two decades[4] and as the population grows older the burden of diabetes and its complications are likely to increase.[5] Saliva is a complex oral fluid which consists of a mixture of secretions from the major salivary glands and the minor glands in the oral cavity.[6] Saliva contains several biochemical components which potentially can be used as diagnostic markers for human disease.[7] Diabetes mellitus is reported to be associated with altered salivary composition and functions. Diabetes mellitus disrupts the homeostasis of the oral cavity and makes it susceptible to various oral disorders.[8] Hence the possible utility of saliva as a diagnostic bio-fluid have led many researchers to develop saliva based technology to detect the transition between health and disease.[9] Saliva has multiple potential advantages over blood testing. Saliva collection being non-invasive and safer can be performed by the patients at home and delivered to nearby facilities.[10] Hence, the present study was designed at People's College of Dental Sciences and Research Centre, Bhopal, Madhya Pradesh to correlate the serum and salivary glucose levels in diabetic and healthy individuals, and determine salivary flow rate, pH and buffering capacity among the study subjects.

MATERIALS AND METHODS

The present study was designed with a sample size of 80 patients. Ethical clearance was obtained from the ethical committee of the institute. Study protocol was explained to patients and oral as well as written consent was obtained from all the patients.

The study comprised of 3 Groups. Group I and Group II consisted of diabetic patients. Group III comprised of healthy individuals.

Inclusion criteria was based on the criteria as per established by the Expert committee on diagnosis and classification of diabetes mellitus in 1998(I). Patients selected were established cases of diabetes mellitus diagnosed by the medical faculty with features of polyuria, polydypsia and polyphagia and elevated blood glucose levels.

Study Group

Group I: consisted of 30 uncontrolled diabetic patients. They were type 2 diabetics with uncontrolled metabolic state and were not taking any drugs other than those to control diabetes.

Group II: comprised of 30 controlled diabetic patients. They were type 2 diabetics with their metabolic state under control. They were on oral hypoglycaemic and not taking any other medication.

Group III: consisted of 20 healthy non-diabetic subjects with no features of diabetes mellitus and blood glucose levels were within normal limits.

Exclusion criteria were patients with severe diabetic complications, with any other systemic illnesses or on medications other than those for diabetes. Patients with habits of smoking, alcoholism and denture wearers were excluded.

Blood glucose level was used as an indicator of metabolic control to differentiate the patients into controlled diabetics and uncontrolled diabetics: Diabetic patients having fasting blood glucose level <140 mg/dl and Postprandial blood glucose level <200 mg/dl were grouped as controlled diabetics. Diabetic patients having fasting blood glucose level ≥140 mg/dl and Postprandial blood glucose level ≥200 mg/dl were grouped as uncontrolled diabetics.

Sample Collection

Blood and saliva sample collection were done simultaneously**.** Blood was collected by intravenous blood sampling method. Unstimulated saliva

was collected by the spitting method in sterile graduated sample collection container for at least 5 min in the morning between 8-11 am to avoid circadian variations. The collected samples were stored until analyzed.

Biochemical Analysis

Blood and salivary samples were immediately transported to the laboratory and were analyzed on the same day. Samples were first centrifuged at 3,000 rpm for 5-10 min and clear supernatants were processed immediately for estimation of glucose. Glucose was estimated in the supernatant saliva and serum by the glucose oxidase-peroxidase method.[7,11-14] 1,000 µl of reagent solution was pipetted into each of the 3 test tubes labelled 'Blank', 'Standard' and 'Test'.10 µl of standard was added to the test tube labelled as 'Standard', followed by 10 µl of test sample to the 'Test' test tube. These were mixed well and all the test tubes were kept in an incubator at 37°C for 10 min before aspiration. Firstly, reagent blank was aspirated in the semi-automated analyser, followed by standard solution, for which the reading was noted, and finally, the test sample was aspirated and the reading was noted. The results were calculated and values are expressed as milligrams per decilitre (mg/dl).[14]

Unstimulated salivary flow rates (USR) were measured in terms of saliva expectorated in milliliter per minute (ml/min) in all the patients. The pH of unstimulated saliva was estimated by dipping the pH test paper directly into the salivary sample. The buffering capacity of unstimulated saliva was assessed using buffer powders.

Statistical analysis

Database management and all statistical analysis were performed with the Statistical Package for the Social Sciences (SPSS version 20) software. One-way ANOVA was used to test for differences between the means of the three groups. Relationships between the variables were evaluated by Pearson correlation coefficient. A P value of <0.05 was considered to be statistically significant.

RESULTS

The mean serum glucose was significantly higher in group I (280.43 ± 69.7 mg/dl) as compared to group II (155.83 ± 11.4 mg/dl) and group III (96.150 ± 17.2 mg/dl) (P<0.001). The mean salivary glucose levels were significantly higher in diabetic subjects compared to non-diabetic subjects. Similar to the serum glucose levels, the salivary glucose levels in group 1 was significantly higher than group II and III (Table 1).

Salivary glucose levels positively and significantly correlated with serum glucose levels in all the groups (Figure 1, Table 2 and 3).

The comparison of salivary flow rate among the three groups was found to be statistically significant (Figure 2).

Salivary glucose and salivary flow rate was negatively correlated in all the groups by pearson correlation coefficient test and was found to be statistically significant in all the three groups.

In Group I, a statistically significant correlation was observed between salivary glucose and salivary flow rate (Figure 3).

The mean pH values in group I (6.10 ± 1.67), group II (5.47 ± 1.22) and group III (6.9 ± 1.83) were similar. The buffering capacity among the 3 groups was also normal and similar among the three groups.

DISCUSSION

The mean salivary glucose levels (1.380 ± 0.516 mg/dl) in non-diabetic subjects were higher in the present study compared to other studies,[15,16] which could be attributed to the carbohydrate-rich dietary pattern of the Indian population. Salivary glucose levels were significantly higher in diabetic subjects (group I>group II) than in non-diabetic subjects (group III). Salivary glucose levels showed significant positive correlations with serum glucose levels in all the study groups. However a previous study reported such positive correlation only in the group with uncontrolled diabetes.[14] While fasting salivary glucose levels in diabetic patients is also reported to positively correlate with serum glucose levels.[12] Thus the salivary glucose levels closely reflect blood glucose levels and can be used as a reliable non-invasive tool to monitor glycaemic control in diabetic subjects.

Various investigators have reported decreased salivary flow rate in diabetic subjects.[17-19] In our study salivary flow rates were significantly decreased in diabetic subjects compared to nondiabetic subjects. Previously lower salivary flow rate is reported in type 2 diabetic patients compared with healthy individuals.[20] A statistically significant correlation was observed between salivary flow rate and the concentration of salivary glucose in our study, which is consistent with a previous study.[21] The mean salivary pH was similar in all the groups and all studied individuals exhibited good buffering capacity.

An integral part of diabetes mellitus therapy is self-measurement of glucose.[22] Hence most patients are anxious for less invasive methods for glucose measurement, this may be achieved using saliva as a biological sample to measure glucose levels.[22] Moreover several other components

Table 1: Comparison of salivary glucose among the three groups

Parameter	Group	Mean	SD	N	F Value	P Value
Salivary glucose (mg/dl)	Group I	21.93	4.53	30	353.3	<0.0001
	Group II	4.73	2.02	30		
	Group III	1.38	0.516	20		

Table 2: Comparison of salivary and serum glucose in controlled diabetics (Group II)

Group	Parameters	Mean Reduction	SD	N	r Value	P Value
Group II	Serum glucose	155.83	11.4	30	0.65	<0.0001
	Salivary glucose	4.73	2.02	30		

Table 3: Comparison of salivary and serum glucose in non-diabetics (Group III)

Group	Parameters	Mean Reduction	SD	N	r Value	P Value
Group III	Serum glucose	96.150	17.2	20	0.80	<0.0001
	Salivary glucose	1.38	0.516	20		

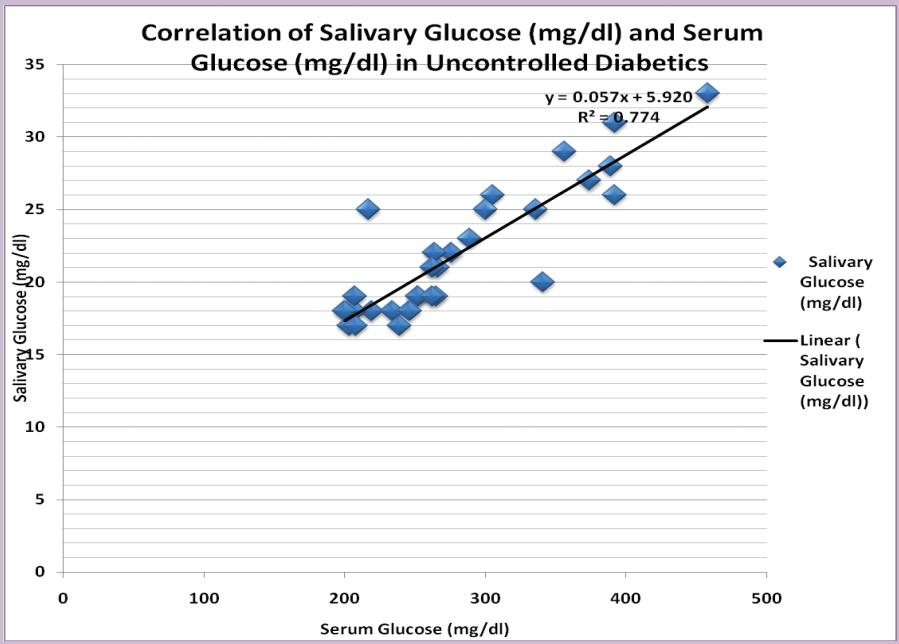

Figure 1: Correlation of salivary glucose and serum glucose in uncontrolled diabetics (Group I).

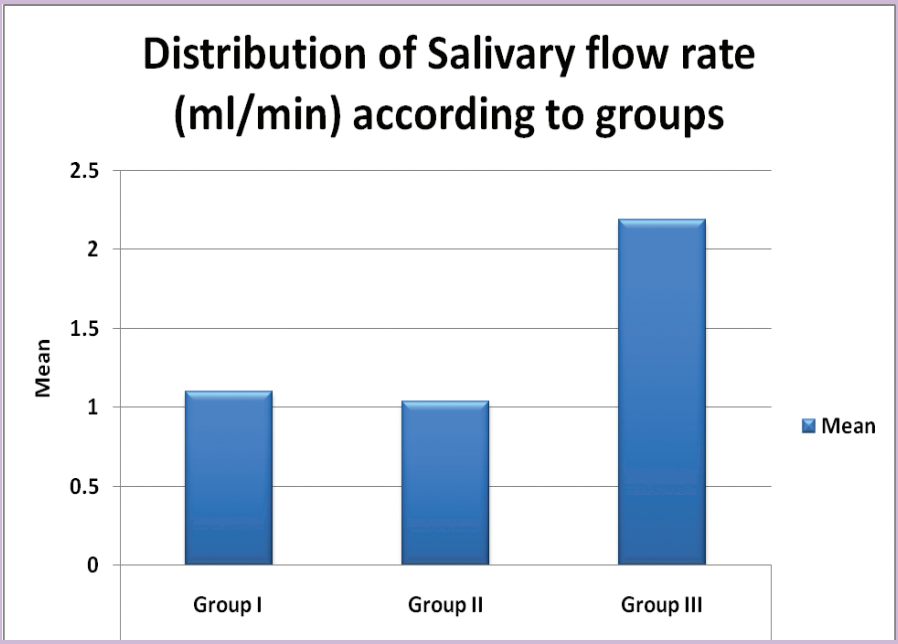

Figure 2: Comparison of salivary flow rate among the three groups.

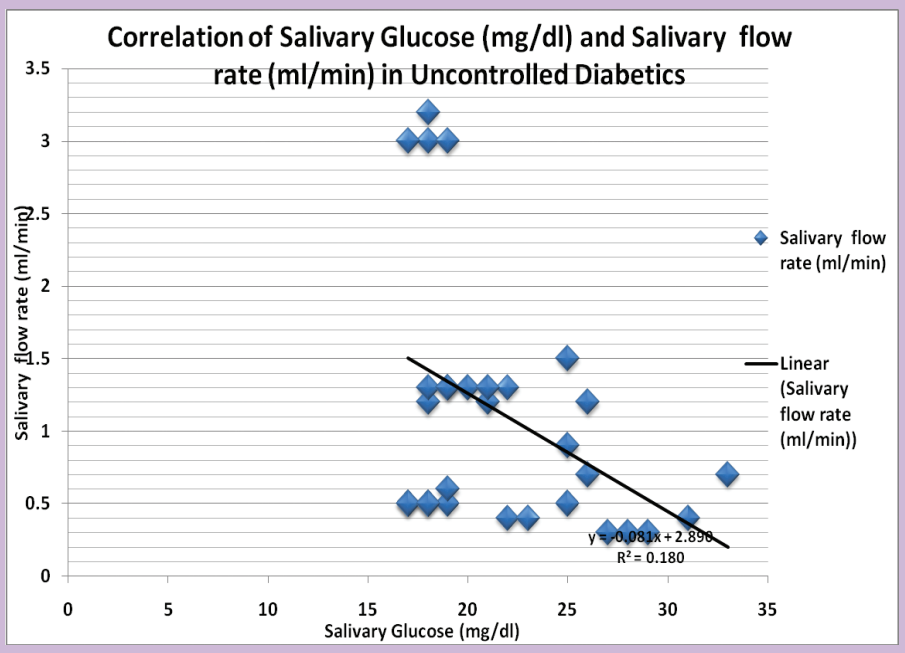

Figure 3: Correlation of salivary flow rate and salivary glucose in Group I.

of saliva may be potential indicators of oral or systemic alterations. Hence saliva may be a potential substitute for blood in lab tests for the diagnosis of some illnesses,[21] including diabetes mellitus. In this context our study supports the use of saliva as a diagnostic fluid in diabetes where it would especially prove valuable in improving patients compliance in self-monitoring the disease control.

CONCLUSION

The present findings show that in diabetic subjects, salivary glucose levels have a significant positive correlation with blood. Thus, salivary glucose levels could be a potentially useful non-invasive tool to monitor glycaemic control in diabetic patients.

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CONFLICTS OF INTEREST

The author declare no conflict of interest.

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