



UTILIZATION OF SALIVARY GLUCOSE FOR MONITORING DIABETIC STATUS

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ABSTRACT

Background: Diabetes is a silent epidemic disease which affects a larger population around the world. It occurs as a result of insulin deficiency or resistance of cells to insulin. India stands seconds in total number of population affected with diabetes following China which is first in position.

Aim: (1) To assess the presence of glucose molecule in saliva similar to that of blood. (2) To estimate the serum and salivary glucose levels by colorimetric method in diabetics and comparing it with healthy subjects.

Study design: A total of 180 subjects were included in the study dividing them into three groups - Normal subjects and diabetic groups were divided into controlled and uncontrolled diabetics based on HbA1c values. (60 subjects in each group).

Materials and Methods: Estimation of serum and salivary glucose was carried out based on GOD-POD principle. The HbA1c values were obtained by WB / Iron – Exchange HPLC method.

Results: Positive correlation between fasting serum glucose and salivary glucose in group I, II and III which was found to be statistically significant ($p<0.05$). Positive correlation in controlled diabetics(0.2493)and Uncontrolled diabetics (0.3735) was found between post prandial serum glucose and post prandial salivary glucose, which is statistically significant ($P<0.05$).

Conclusion: Glucose level in saliva and serum of healthy subjects, controlled and uncontrolled diabetics were found to alter according to the serum glucose level. This proves that saliva can be used as a diagnostic tool in monitoring the diabetic status.

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INTRODUCTION

Diabetes is one of the most rapidly growing silent epidemic disease that has the capacity to affect larger population around the world. It is a chronic disease which usually occurs due to insulin deficiency or resistance of cells to insulin which leads to state of hyperglycaemia and other disorders of metabolism.¹ As on 2011, 366 million people worldwide were diabetic and the number of people affected are steadily increasing. At present, India is the second country in the list of people affected with diabetic following China which is in first position.² In the present scenario, the detection of diabetes is done by estimating blood glucose levels and glycated haemoglobin (HbA1c) levels in serum.¹ Blood is the usual diagnostic medium used for biochemical analysis, similarly saliva can also be used as it also reflects the changes occurring in the blood.³ Glucose, a biochemical sugar molecule that easily

permeates through the membrane of the blood vessels and reaches the saliva from the gingival fluid and analysis of this glucose molecule in saliva may be helpful in monitoring the diabetes status.⁴ Saliva can be collected by a non – invasive method and offers a great benefit in situation where whole blood drawing is extremely difficult like in obese and haemophilic patient and lacks clotting tendency.⁵ Since elevated blood glucose levels were reflected in the saliva as high salivary glucose levels,⁶ saliva provides a promising role as an alternative medium for screening and diagnosing diabetes.⁷

So the study was aimed to find out whether saliva can be used to diagnose and monitor the diabetes status and also to estimate the serum and salivary glucose levels by colorimetric method in diabetics and comparing it with healthy subjects.

MATERIALS AND METHODS

A total of 180 subjects were included in this study with 60 subjects in each group and they were categorized as Group I (Healthy subjects), Group II (Controlled diabetic subjects - HbA1c value: 6.5 to 7.9 %), Group III (Uncontrolled diabetic

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subjects - HbA1c value $\geq 8\%$). The diabetic groups were divided based on their HbA1c values. Written consent was obtained from the subjects participating in the study. The study was approved by Institutional Review Board.

Sample Collection

Subjects who met the inclusion criteria were selected from the dental outpatient department and those attending general hospital. Patients were asked to report in the morning to outpatient department in empty stomach after a period of 8 hours of fasting. The subjects were explained about the procedure to be carried out.

Blood Collection

2 ml of only fasting blood sample was collected using a sterile disposable syringe from healthy subjects and stored in a sterilised glass tube and 2 ml each of fasting and post prandial blood sample were collected using a sterile disposable syringe from diabetic subjects. 2 ml blood sample was also collected for HbA1c estimation from diabetic subjects only.

Saliva Collection

Saliva samples were collected from healthy subjects (only fasting) and diabetic subjects (fasting and post prandial) after asking the subjects to rinse the mouth with tap water. Then the subjects were asked to spit the saliva pooled for following 10 minutes into a sterile container. The Blood samples, saliva containers and EDTA tubes were labelled with subject details including subjects name, age and sex.

METHODOLOGY

Estimation of glucose in serum and saliva was done following the principle of Glucose oxidase peroxidase method. The blood and saliva sample collected are centrifuged in the centrifuge machine at 3000 rpm for about 10 minutes. The serum and supernatant saliva is used for biochemical investigation. Three test tubes containing 50 μ l of reagent is taken and marked as B (Blank), S (Standard) and T (Test) test tubes.

Test tube B contains 5 μ l of Deionized water, test tube S contains 5 μ l of standard glucose solution, and test tube T contains 5 μ l of serum / saliva sample. All the three test tubes were incubated for 5 min at 37°c temperature. The colorimeter was used to read the glucose values. The HbA1c values were obtained by WB / Iron – Exchange HPLC method.

The values obtained for serum and salivary glucose were analysed for mean, standard deviation, range, correlation.

RESULTS

Table 1 Correlation between Fasting Serum and Salivary glucose levels: Healthy subjects -Group I

Variables	Range (mg/dl)	Mean (mg/dl)	SD (\pm)	r-value
Serum Glucose	64 – 138	91.35	13.76	0.1668 (P < 0.05)
Salivary Glucose	10 – 49	21.6333	8.02	(Significant)

Group I showed serum glucose ranging from 64 – 138 mg/dl with a mean value of 91.35 mg/dl and a SD of 13.76 and similarly Group I showed salivary glucose ranging from 10 – 49 mg/dl with a mean value of 21.6333 mg/dl and a standard deviation of 8.02. Positive correlation (0.1668) was found

between serum glucose and salivary glucose, which is statistically significant ($p < 0.05$), as shown in Table 1.

Table 2 Correlation between Fasting, Post Prandial Serum and Salivary glucose levels :Controlled Diabetic subjects -Group II

Variables	Range (mg/dl)	Mean (mg/dl)	SD (\pm)	r-value
SerumGlucose (F)	86 – 354	141.5	53.56	0.0942 (P < 0.05)
Salivary Glucose (F)	13 – 54	24.9	7.00	(Significant)
Serum Glucose (PP)	116 – 520	213.2	76.47	0.2493 (P < 0.05)
Salivary Glucose PP)	17 – 69	35.08	8.57	(Significant)

Group II showed fasting serum glucose ranging from 86 – 354 mg/dl with a mean value of 141.5 mg/dl and a standard deviation of 53.56 and similarly fasting salivary glucose ranging from 13 – 54 mg/dl with a mean value of 24.9 and a standard deviation of 7. Positive correlation(0.0942) was found between fasting serum glucose and fasting salivary glucose, which is statistically significant ($P < 0.05$) as shown in Table 2.

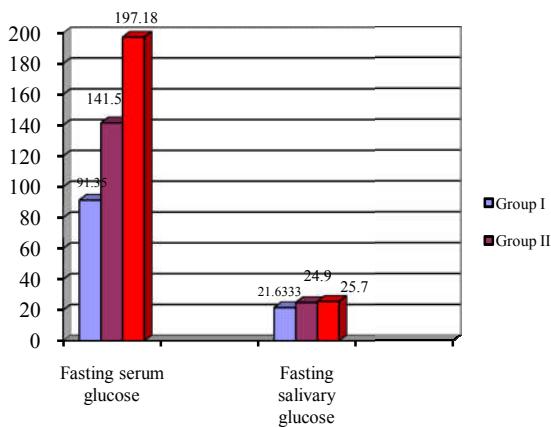
Group II showed post prandial serum glucose ranged from 116 - 520 mg/dl with a mean value of 213.2 mg/dl and a standard deviation of 76.47 and similarly post prandial salivary glucose ranged from 17 – 69 mg/dl with a mean value of 35.08 mg/dl and standard deviation of 8.57. Positive correlation(0.2493) was found between post prandial serum glucose and post prandial salivary glucose, which is statistically significant ($P < 0.05$) as shown in Table 2.

Table 3 Correlation between Fasting, Post Prandial, Blood and Salivary glucose levels: Uncontrolled diabetic subjects- Group III

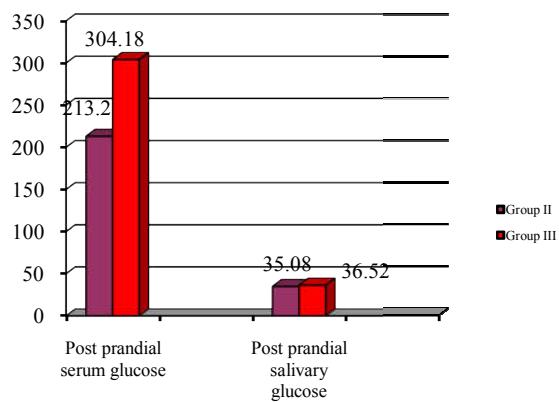
Variables	Range (mg/dl)	Mean (mg/dl)	SD (\pm)	r-value
Serum Glucose (F)	95 – 368	197.18	56.81	0.3299 (P < 0.05)
Salivary Glucose (F)	15 – 41	25.7	5.52	(Significant)
Serum Glucose (PP)	145 – 495	304.18	77.77	0.3735 (P < 0.05)
Salivary Glucose (PP)	19 – 59	36.52	7.53	(Significant)

Group III showed fasting serum glucose ranged from 95 - 368 mg/dl with a mean value of 197.18 mg/dl and a SD of 56.81 and similarly fasting salivary glucose ranged from 15 - 41 mg/dl with a mean value of 25.7 mg/dl and a SD of 5.52. Positive correlation was found between fasting serum glucose and fasting salivary glucose, which was statistically significant ($P < 0.05$) as shown in Table 3.

Group III showed Post prandial serum glucose ranged from 145 - 495 mg/dl with a mean value of 304.18 mg/dl and a SD of 77.77 and similarly postprandial salivary glucose ranged from 19 – 59 mg/dl with a mean value of 36.52 mg/dl and standard deviation of 7.53. Positive correlation was found between post prandial serum glucose and post prandial salivary glucose, which was statistically significant ($P < 0.05$) as shown in Table 3.



Graph 1 Fasting serum and salivary glucose levels - Group I,II& III



Graph 2 Post Prandial serum & salivary glucose -Group II & III

DISCUSSION

Diabetes mellitus is a widely spreading disease in the current global scenario. Various studies conducted in India have shown high prevalence of type II diabetes.⁸ India holds the first position in the list of diabetic prevalence followed by China, USA and Indonesia. So India is designated as the diabetes capital of the world.⁹

Many diagnostic instruments are readily available in the medical care to analyse the blood glucose level but most of these methodologies utilises blood as the diagnostic medium or fluid whose collection is invasive and causes psychological trauma to the patients.¹⁰ So a protocol to determine glucose level in body with an alternate medium utilising non-invasive technique , less cost effective which will be well accepted by the patient. Hereby saliva can fulfil these criterias and serve as a very good alternate medium⁸ as it is considered as the mirror of the body. Oral fluid sampling which is cost effective and safe for both the operator and the patient.¹¹

To the best of our knowledge and extensive search in literature, only few studies analogous to the present study have subdivided diabetics based on HbA1c values [Dr.Lekaa.M.Ibrahem *et al* (2007)]¹².and Preethi Balan *et al* (2014)¹³ and only few estimated postprandial salivary glucose level. [Veena V. Naik *et al* (2011)¹⁴, Dr.Seema Hallikerimath (2011)¹⁵] but did not subdivide there diabetic groups as done in present study.In the present study glucose was detected in the healthy, controlled and uncontrolled diabetic subjects which was in accordance with the studies conducted by

Sreedevi *et al* (2008)⁸, Panda Abikshyeet *et al* (2012)¹, Agrawal RP *et al* (2013)¹⁶, Young (1941)¹⁷, Gheena *et al* (2011)¹⁸, Dipti Soni Jaipuriar *et al* (2016)¹⁹. Positive correlation coefficient between fasting serum glucose and salivary glucose of group I, II and III which was found to be statistically significant ($p<0.05$) in the present study and was in accordance with various authors.^{1,8,17,18}

The estimation of post prandial serum and salivary glucose was also carried out in Group II & III. Our study results was in accordance with results of Veena V. Naik *et al* (2011)¹⁴ and in discordance with that of Dr.Seema Hallikerimath (2011)¹⁵ reason said by the author was that 68% of diabetic subjects were on medication for a longer period contributing to the lesser post prandial salivary glucose levels.

CONCLUSION

Saliva reflects the changes happening in the body fluids especially blood. Though saliva collection is non-invasive, less expensive, easy, even it has few limitations. The presence of glucose in saliva is a multifactorial event involving various factors. Inspite of above said limitations, saliva stands out to be a valid diagnostic and convenient tool for monitoring diabetic status and can mirror the changes happening in the blood.

Though our study results regarding salivary glucose were promising and reflected the blood changes, further studies with larger sample size including subjects representing different geographic locations, different age ranges, different types of diabetes and subjects with varied dietary patterns should be carried out to substantiate that saliva can be an alternate medium compared to blood in the diagnosis and monitoring of diabetic status.

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