Comparative Study between Saliva and Serum in Diabetic Patients

Tanoj Kumar[1], Jay Kishore[2], Ravi Kumar[3]

ABSTRACT

Introduction: Diabetes mellitus is a common chronic metabolic disorder which affects millions of people. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and may reach 4.4% by 2030. The objective of my study was to evaluate and compare thesalivary and serum glucose levels in diabetes mellitus individuals.

Material and methods: Study included 200 subjects, out of these 100 subjects were of diagnosed diabetes & 100 non-diabetic subjects were as controls group. 2ml of patient's intra venous blood was obtained from the median cephalic vein of the forearm, by using 5 ml disposable syringe. The blood sample was transferred in a fluoride tube.

Results: Glucose was present in saliva of both diabetic and non diabetic subjects. Glucose level was increased in diabetic patients.

Conclusion: Concentration of glucose in saliva increases with the increase in serum glucose concentration. A significant correlation was seen between salivary and serum glucose level in diabetic as well as non diabetic subjects.

Keywords: Diabetes, Serum, Saliva

INTRODUCTION

The prevalence of diabetes has increased rapidly during the past several decades and is expected to continue to rise. A consistent finding of this growing epidemic has been that 35% to 50% of cases of diabetes are undiagnosed.[1]

Presenting Symptoms of Diabetes are Polyuria, Polydipsia and Nocturia. Acute hyperglycemia causes increased urine excretion (polyuria) and a result, excessive thirst and water ingestion. These presenting symptom of diabetes mellitus are also termed osmotic symptoms.[2]

It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, the old and infant and in large-scale screening and epidemiological studies. Indeed, all steroids of diagnostic significance in routine clinical endocrinology can now be readily measured in saliva. Tests based on saliva have already made substantial inroads into diagnosis. For some molecules – for example, antibodies, unconjugated steroids, hormones and certain drugs – the techniques are sufficiently sensitive to reflect blood concentrations of the substance accurately.[3]

Aim of this study was to find out the usefulness of saliva in diagnosis of diabetes and whether it can act as adjunct to blood test or can be done with as accurate precision as the blood glucose.

Study aimed to compare the salivary and serum glucose levels in diabetes mellitus patients. With the objectives to compare salivary and serum glucose level in diabetes mellitus individuals and to compare salivary and serum glucose level in nondiabetes mellitus individuals.

MATERIAL AND METHODS

This study was conducted among individuals attending in oral and maxillofacial pathology department, Patna Dental College and Hospital, Patna. Study included 200 individuals, out of these 100 individuals were of diagnosed diabetes S& 100 non-diabetic individuals were as controls group. Mouth mirror, Gloves, mouth-masks, Disposable syringes,Fluoride bulb, GOD-POD kit, Micro-pipette, Centrifuge machine & Semiautoanalyzer machine were used in the study (fig.1 and 2).

Method of collection of saliva sample

- The patient was asked to rinse his/her mouth thoroughly.
- After 3-4 minutes, 2ml of unstimulated saliva was collected in a fluoride tube by drooping method.

Method of collection for serum sample

2ml.of patient's intra venous blood was obtained from the median cephalic vein of the forearm, by using 5 ml disposable syringe. The blood sample was transferred in a fluoride tube.

Method of test of saliva & blood

The glucose estimation kit was first reconstituted. The working reagent in powder form is dissolved in the glucose diluent and stored in a clean and dry amber coloured bottle. The reconstituted reagent is stable for at least 90 days at 2 - 8 ^{0} C. The sample collected (saliva/blood) was centrifuged in the centrifuge machine (fig.1). The supernatant was used to estimate the glucose. Three separate sterile test tubes are taken and 1000 ml of working reagent was taken in each of them. Then to the first test tube 10 ml of Deionized water was added. This is known as blank. And in the second test tube 10 ml of Standard glucose solution which contains 100 mg/dl of glucose was added. This is known as Standard. In the third test tube 10 ml of sample (saliva/serum) was added. This is known as Test. The content of all the three test tubes

[1]Professor, Department of Oral and Maxillofacial Pathology, Patna Dental College and Hospital, Patna, [2]Senior Resident, Department of Dentistry, Shri Krishna Medical College and Hospital, Muzaffarpur, Bihar, [3]Assistant Professor, Department of Dentistry, Heritage Institute of Medical Sciences, Bhadwar, Varanasi, India

Corresponding author: Jay Kishore, 8L-30, Bahadurpur Housing Colony, Patna- 800026, India

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were mixed well and incubated for 15 minutes at 37 ^{0} C. The semi auto analyzer (accurex company) is used to read the absorbance. First the absorbance of blank was measured followed by the standard. The analyzer is pre-programmed to give the value of glucose content of standard which is used as the baseline to estimate the glucose content of test. When the absorbance of test is measured, the analyzer automatically gives the glucose content of the test.

RESULTS

Table 1 shows that 64 male & 36 female in diabetic group individuals, and 72 male & 28 female in non diabetic group individuals.

The study group was comprised of 100 diabetic subjects & 100 non-diabetic (control) subjects. Among Diabetic subjects, 17 (17%) subject were 20-30 years of age group while 21 (21%) were 31-40 yrs of age group, while 18 (18%) were 41-50 age group, 44 (44 %) subjects were more than 50 years of age groups. Among control group, 61 (61%) subject were 20-30 years of age group while 22 (21%) were 31-40 yrs of age group, while 4 (4%) were 41-50 age group, 13 (13 %) subjects were more than 50 years of age groups (table 2). Among Diabetic subjects, out of 100 subjects, 24 had a PBS value between 101-200 mg/dl with 10.833±2.0816 PSS level, 60 subjects had a PBS value between 201-300 mg/dl with a mean value of 1.167±1.555 PSS level & 16 subjects had a PBS value between >300 mg/dl with a mean value of 16.3750±2.352 PSS level (table 3). Table 4 shows correlation of serum and salivary glucose levels among the various study group.

Figure-2 & 3: Armamentarium used, GOD-POD Kit & Micropipettes

Figure-1: Showing centrifugation machine

Study groups	Male	Female
Diabetic	64	36
Non diabetic	72	28

Table-1: Gender Distribution of Patients in Study Group

Study groups	20-30	31-40	41-50	>50
Diabetic	17	21	18	44
Non diabetic	61	22	4	13

Table-2: Age Distribution of Patients in Study Group

DISCUSSION

Saliva indeed is a mirror of our blood as these bio-fluids and their molecular components share many similarities. Realization of this fact and the possible utility of saliva as a diagnostic bio-fluid during the past couple of decades have led many researchers to develop saliva-based technology to detect the transition between health and disease. Furthermore, recent advances in technology including genomics, proteomics, transcriptomics and microfluidics have led the way for using saliva beyond basic assessment of oral health characteristics to where it could be used to evaluate features of overall health including disease progression. Recent research has now established defined ways to work with saliva that are consistent, reproducible, and which keep these molecules in an un-degraded, stable state.

In order for a diagnostic method including salivary diagnostics to be widely applicable it should satisfy the

PBS (mg/dl)	No. of Patients	PSS						
		Mean	Standard deviation	Standard Error	95% Confidence interval for mean		Minimum Value of PSS	Maximum Value of PSS
					Lower bound	Upper bound		
101-200	12	10.8333	2.08167	.60093	9.5107	12.1560	9.00	15.00
201-300	30	13.1667	1.55549	.28399	12.5858	13.7475	11.00	16.00
>300	8	16.3750	.91613	.32390	15.6091	17.1409	15.00	18.00
Total	50	13.1200	2.35294	.33276	12.4513	13.7887	9.00	18.00

Table-3: Mean & Standard Deviation of PSS In Various PBS Grades among Type I Diabetic Patient.

Study groups	Correlation coefficient	p-value	Significance
Control	0.630	0.000	HS
Diabetes	0.824	0.000	HS

Table-4: Correlation of Serum and Salivary Glucose Levels among the Various Study Group

following criteria:

- Analytic precision and accuracy
- Excellent sensitivity and specificity
- Good operational predictive value and efficiency
- Value for money or cost/benefit ratio in terms of rapid point of care diagnosis.

Saliva can be collected with non invasive technique without any discomfort to the patient.

The evaluation of secretion from individual glands is useful in detection of gland`s specific pathology. While saliva being a mixture of whole fluid, secretions of all salivary glands, expectorated secretions, microorganisms & their products, desquamated epithelial cells and food debris are usually analyzed for detection of systemic disorders.

Unstimulated saliva was collected by draining method 2 hrs after meal. About 2ml of saliva was collected for sugar concentration evaluation.

It is a normal tendency to avoid the prick. Considering the fact that frequent monitoring of blood glucose levels is necessary in diabetic patients, it is always disheartening and a source of constant annoyance to the patient. A non-invasive, simple and painless procedure like salivary glucose estimation is very much desirable in this scenario.

Our study estimated the post-prandial salivary glucose level in diabetic patients and control subjects. The purpose was to determine if any significant correlation was present between the post prandial blood glucose level and salivary glucose level in type I diabetes, type II diabetes, newly diagnosed diabetes & in control group, whether any difference is noticed between the study and the control group, and whether salivary glucose level can be used as a diagnostic adjunct for diabetes.

Collected sample were immediately analyzed to avoid deterioration of sample by enzymatic alteration of sugar content in saliva.

The glucose analysis of both serum and saliva was done by the GOD-POD method.

In the present study, we included a total of 200 subjects, out of which 100 were diagnosed diabetic patients & 100 subjects in Control Group. For each individual participating in this study, post prandial glucose concentration estimation in saliva & serum was done. All the subjects included in our study were informed about the study.

In our study we noted that glucose concentration was present in saliva of diabetic as well as non diabetic subjects. Fleckseder and Carlson et al[4] reported the presence of sugar in the saliva of diabetic patients. We noted that the post prandial salivary glucose values were higher among the diabetics than in controls. We also noted that, with the increase in glucose concentration in serum there is increase in salivary glucose concentration.

Reuterving et al,[5] Ben-Aryeh et al,[6] Shehla Amer et al[7] Sreedevi et al,[8] all reported increase in salivary glucose level in patients of diabetes mellitus in comparison to non diabetics. In our study, correlation between salivary glucose and serum glucose in diabetics and controls was carried out based on Pearson's correlation coefficient. There was correlation between salivary and serum glucose in diabetic patients. In controls also, correlation between salivary glucose and serum glucose was found. These correlations were found out to be statistically significant. Hence, salivary glucose appears to be an indicator of serum glucose concentration in diabetic patients.

Similar to our study, Reuterving et al,[5] too found a positive correlation between salivary glucose and serum glucose. In our study we found that out that salivary glucose level increases with increase in serum glucose level. We observed that, patients having a serum glucose level below 140 mg/dl showed a salivary glucose level of 4-8 & patients having serum glucose level between 140 – 200 mg/dl showed a salivary glucose of 8-10 mg/dl, and patients having serum glucose level above 200 mg/dl reflected a mean salivary glucose level of 9-13 mg/dl.

In our study we could not find any difference in salivary glucose when we compared it with different age groups & there was slightly male predilection. Salivary glucose concentration was found directly proportional to serum glucose concentration. These findings were consistent with the findings by Sreedeviet al.[8]

Englander et al,[9] Thorstensson et al,[10] reported increase in salivary glucose level in patients of diabetes mellitus in comparison to non diabetics. In our study, correlation between salivary glucose and serum glucose in diabetics and controls was carried out based on Pearson's correlation coefficient. There was correlation between salivary and serum glucose in diabetic patients

CONCLUSION

Glucose was present in saliva of both diabetic and non diabetic subjects. Concentration of glucose in saliva increases with the increase in serum glucose concentration and vice versa. A significant correlation was seen between salivary and serum glucose level in diabetic as well as non diabetic subjects. There was no correlation between salivary or serum glucose concentration with age & sex. Salivary glucose level reflects the level of glycemic achieved in a patient of diabetes mellitus.

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