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ORIGINAL RESEARCH

Evaluation of the Salivary and Serum Glucose Levels in Diabetes Mellitus Patients: A Comparative Study

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ABSTRACT

Background and objectives: Diabetes mellitus is a common chronic metabolic disorder that 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. Additionally, the diabetic population is expected to rise from 171 million in 2000 to 3666 million by 2030. Recent World Health Organization calculations indicated that worldwide almost 3 million deaths per year are attributed to diabetes, equivalent to 5.2% of all deaths. The objective of this study is to evaluate and compare the salivary and serum glucose levels in diabetes mellitus individuals.

Materials and methods: Two milliliter saliva and serum samples were collected. Both samples were centrifuged at 2000 rpm for 2 to 3 minutes. With the help of a micropipette, 1 ml of the glucose reagent was taken into the other test tube. Then 10 µl of the supernatant sample was taken out and added to the glucose reagent. This was kept in a temperature-controlled water bath at 37°C for 10 minutes. The color change was noted and the optical density was measured in a semiautoanalyzer.

Results: Glucose is present in saliva of both diabetic and non-diabetic subjects. Glucose level increases 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 nondiabetic subjects.

Keywords: Diabetes, Saliva, Serum.

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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]

Diabetes mellitus is a common chronic metabolic disorder that 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. Additionally, the diabetic population is expected to rise from 171 million in 2000 to 3666 million by 2030. Recent World Health Organization calculations indicated that worldwide almost 3 million deaths per year are attributed to diabetes, equivalent to 5.2% of all deaths.[2]

Presenting symptoms of diabetes are polyuria, polydipsia, and nocturia. Acute hyperglycemia causes increased urine excretion (polyuria) and as a result excessive thirst and water ingestion. These presenting symptom of diabetes mellitus are also termed "osmotic symptoms."[3]

Many people with type II diabetes may be asymptomatic at diagnosis, e.g., by routine screening of blood or urine, when they may be only mildly hyperglycemic. Once diagnosed, however, many patients do admit to some long-standing, often mild symptoms. Acute metabolic decompensation, leading to marked hyperglycemia, occurs frequently.[4]

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 infants 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.[5]

Replacing blood draws with saliva tests promises to make disease diagnosis, as well as the tracking of treatment efficacy, less invasive and costly.[6]

An early diagnosis and effective treatment is critical for preventing the disease's complications, including loss of vision, nerve damage, and kidney damage. One important barrier is the need for sometimes-painful needle sticks to draw blood for tests. The discomfort can discourage patients from properly monitoring their blood sugar levels, scientists say.[6]

Now the present concept is to find out a medium that can be used to diagnose and monitor diabetes and here saliva can play a major role. Saliva is one of the most abundant secretions in the human body, which can be very easily obtained. The reason is that it can be collected very easily and the process will be noninvasive.[6]

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

AIM

To evaluate and compare the salivary and serum glucose levels in diabetes mellitus individuals.

OBJECTIVES

- To evaluate the salivary glucose level in diabetic individuals.
- To evaluate the salivary glucose level in nondiabetic individuals.
- To evaluate the serum glucose level in diabetic individuals.
- To evaluate the serum glucose level in nondiabetic individuals.
- To correlate salivary glucose in diabetic and non-diabetic individuals.
- To compare salivary and serum glucose levels in diabetes mellitus individuals.

MATERIALS AND METHODS

Source of Data

This study was conducted among individuals attending Dhiraj General Hospital, Piparia, Vadodara. Prior to the study, written permission was taken from the director of the hospital and the Head of biochemistry laboratory. Voluntary healthy (nondiabetic) subjects without any manifesting disorder were taken as the control group. After explaining the purpose and need of the study, an informed written consent was obtained from each individual. A performa with demographic detail was completed.

Sample Size

The study included 200 individuals, out of which 50 had type I diabetes, 50 had type II diabetes, 50 were newly diagnosed diabetic individuals, and the remaining 50 were nondiabetic individuals who served as the control group.

Materials

- Mouth mirror
- Gloves, mouth masks
- Disposable syringes
- Fluoride bulb
- GOD-POD kit
- Micropipette
- Centrifuge machine
- Semiautoanalyzer machine.

Method of Collection of Saliva Sample

- The patient was asked to rinse his/her mouth thoroughly.
- After 5 minutes, 2 ml of unstimulated saliva was collected in a fluoride tube by drooping method.

Method of Collection of Serum Sample

Two milliliters of patient's intravenous blood was obtained from the median cephalic vein of the forearm, by using a 5 ml disposable syringe. The blood sample was transferred into a fluoride tube.

Method of Test of Saliva and Blood

- The glucose estimation kit was first reconstituted. The working reagent in powder form was dissolved in the glucose diluent and stored in a clean and dry amber-colored bottle. The reconstituted reagent is stable for at least 90 days at 2 to 8°C.
- The sample collected (saliva/blood) was centrifuged in the centrifuge machine. The supernatant was used to estimate the glucose.
- Three separate sterile test tubes were taken and 1000 μl of working reagent was taken in each of them.
- To the first test tube 10 μl of deionized water was added. This is known as blank.
- To the second test tube 10 μl of standard glucose solution containing 100 mg/dl of glucose was added. This is known as standard.

Fig. 1: GOD-POD kit and micropipettes

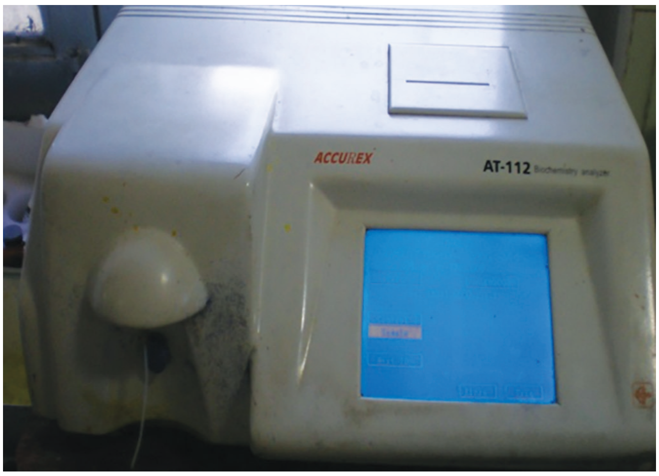

Fig. 2: Semiautoanalyzer machine

- To the third test tube 10 µl of sample (saliva/serum) was added. This is known as test.
- The content of all the three test tubes were mixed well and incubated for 15 minutes at 37°C.
- The semiautoanalyzer (Accurex company) was used to read the absorbance. First the absorbance of blank was measured followed by the standard. The analyzer is preprogrammed to give the value of glucose content of the standard, which is used as the baseline to estimate the glucose content of the test. When the absorbance of the test is measured, the analyzer automatically gives the glucose content of the test.

RESULTS

Table 1 shows the mean and standard deviation of PSS and PBS for type I diabetic patients.

Among the 50 type I diabetic subjects, 12 had a PBS value between 101 and 200 mg/dl with a mean value of 10.833 ± 2.0816 PSS level, 30 had a PBS value between 201 and 300 mg/dl with a mean value of 1.167 ± 1.555 PSS level, and 8 had a PBS value > 300 mg/dl with a mean value of 16.3750 ± 2.352 PSS level. Among the type II diabetic patients, all 50 had 13.12 ± 2.352 PSS level.

Table 2 shows the mean and standard deviation of PSS and PBS grades among newly diagnosed diabetic patients.

Among the 50 newly diagnosed diabetic patients, 44 had a PBS value between 201 and 300 mg/dl with a mean value of 12.8864 ± 1.31566 PSS level and 6 had a PBS value > 300 mg/dl with a mean value of 19.0000 ± 2.44022 PSS level. In newly diagnosed diabetic patients, all 50 subjects had 13.6200 ± 2.44022 PSS level.

Table 3 shows the mean and standard deviation of PBS and PSS value with gender distribution in type II diabetic subjects.

In PBS value, out of 50 subjects, 29 were male with a mean ± SD of PBS of 240.97 ± 68.622 and that of PSS

Table 1: The mean and standard deviation of PSS in various PBS grades among type I diabetic patients

		PSS						
					95% Confidence interval for mean			
PBS (mg/dl)	*No. of patients*	*Mean*	*Standard deviation*	*Standard error*	*Lower bound*	*Upper bound*	*Minimum value of PSS*	*Maximum value of PSS*
101–200	12	10.8333	2.08167	0.60093	9.5107	12.1560	9.00	15.00
201–300	30	13.1667	1.55549	0.28399	12.5858	13.7475	11.00	16.00
>300	8	16.3750	0.91613	0.32390	15.6091	17.1409	15.00	18.00
Total	50	13.1200	2.35294	0.33276	12.4513	13.7887	9.00	18.00

Table 2: The mean and standard deviation of PSS in various PBS grades among newly diagnosed diabetic patients

		PSS						
					95% Confidence interval for mean			
PBS (mg/dl)	*No. of patients*	*Mean*	*Standard deviation*	*Standard error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*
201–300	44	12.8864	1.31566	0.19834	12.4864	13.2864	10.00	16.00
>300	6	19.0000	2.00000	0.81650	16.9011	21.0989	16.00	21.00
Total	50	13.6200	2.44022	0.34510	12.9265	14.3135	10.00	21.00

Table 3: The mean and standard deviation of PBS and PSS value with gender distribution in type II diabetic patients

		Number of patients	Mean	Std. deviation	Std. error	95% Confidence interval for mean Lower bound	95% Confidence interval for mean Upper bound	Minimum	Maximum
PBS	Male	29	240.97	68.62293	12.74296	214.8628	267.0683	178.00	410.00
	Female	21	222.19	59.29386	12.93898	195.2002	249.1807	180.00	397.00
	Total	50	233.08	64.91182	9.17992	214.6323	251.5277	178.00	410.00
PSS	Male	29	12.0000	3.30584	0.61388	10.7425	13.2575	9.00	20.00
	Female	21	10.7143	3.03550	0.66240	9.3325	12.0960	8.00	19.00
	Total	50	11.4600	3.22750	0.45644	10.5428	12.3772	8.00	20.00

of 12.0 ± 3.31, while among the 21 female subjects, the mean ± SD of PBS was 222.19 ± 59.29 and that of PSS was 10.71 ± 3.03. All subjects had a mean ± SD of PBS of 233.08 ± 64.91 and that of PSS of 11.46 ± 3.22.

DISCUSSION

As diabetes is a chronic condition, monitoring of blood glucose at frequent intervals becomes necessary.

Saliva is one of the most abundant secretions in the human body, which can be very easily obtained. Now the present study is to find out a medium that can be used to diagnose and monitor diabetes and here saliva can play a major role. It can be collected with a noninvasive technique without any discomfort to the patient.

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

Many chemical and physical factors are known to influence the composition of mixed saliva. To avoid some discrepancies in the result, patients were asked to rinse their oral cavity properly. This was done to eliminate the chances of food residue providing a source of glucose.

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

At present, the diagnosis of diabetes is achieved only by analyzing the blood glucose levels (random/fasting/post-prandial). But these methods are invasive in nature and are physically as well as psychologically traumatic to the patient. It is a normal tendency in people to avoid a 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 noninvasive, 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, and 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 samples were immediately analyzed to avoid their deterioration 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 50 had type I diabetes, 50 had type II diabetes, 50 had newly diagnosed diabetes, and 50 were subjects in the control group. For each individual participating in this study, post-prandial glucose concentration estimation in saliva and serum was done. All the subjects included in our study were informed about the study. An informed written consent was obtained from every subject included in the study.

In our study we noted that glucose concentration was present in the saliva of diabetic as well as nondiabetic subjects.

Fleckseder and Carlson et al reported the presence of sugar in the saliva of diabetic patients.[7]

In our study we noted that the post-prandial salivary glucose values were higher among the diabetics than among controls. We also noted that with the increase in glucose concentration in serum there is increase in salivary glucose concentration.

Contrary to our findings, Forbat et al concluded that salivary glucose levels did not reflect blood glucose levels.[8] Similarly Carda et al concluded that 76.4% of diabetic patients have their salivary glucose level in the normal range.[9]

Thorstensson et al,[9] reported increase in salivary glucose level in patients of diabetes mellitus in comparison to non diabetics. In our study, a 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[10], Belazi et al[11], Sreedevi et al[12] and Jurysta et al[13] too found a positive correlation between salivary glucose and serum glucose. However in contrast to our study, Forbat et al,[8] could not establish correlation between salivary glucose and serum glucose.[8]

Englander et al expressed doubt regarding replacement of plasma with parotid secretion in the diagnosis of diabetes mellitus because of its lower magnitude of glucose concentration.[14] A saliva analyzing system using a glucose sensor and performed in vivo evaluations and concluded that their salivary glucose level measurement system can be used as an indicator of blood glucose level.

We divided the diabetic patient group into three subgroups based on their serum glucose level and compared the salivary glucose level of the three groups. We found out a statistically significant difference between the three groups and the salivary glucose level was found out to be increasing with increase in serum glucose level. We observed that patients having a serum glucose level below 130 mg/dl showed a salivary glucose level of 5 to 7 mg/dl, patients having serum glucose level between 130 and 200 mg/dl showed a salivary glucose level of 6 to 10 mg/dl, and patients having serum glucose level above 200 mg/dl reflected a mean salivary glucose level of 9 to 11 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 Sreedevi et al.[12]

We all know that glucose is present in the saliva of a normal individual. But the mechanism of its secretion is still obscure. Both a paracellular as well as an intercellular pathway has been proposed. It is not yet an established theory but is still a hypothesis. Many authors tried to explain the increased glucose content in salivary secretion of diabetic patients. Lopez et altried to commit that the salivary glands act as filters of blood glucose that would be altered by hormonal or neural regulation.[15] According to Qureshi et al persistent hyperglycemia leads to microvascular changes in the blood vessels as well as basement membrane alteration in salivary gland.[16] This leads to increased leakage of glucose from ductal cells of the salivary gland and there by increased glucose content in saliva. Sreedevi et al quoting the works of Harrison commented that glucose is a small molecule which easily diffuses through semi permeable membranes.[12] Thus large amount of glucose becomes available to saliva when blood glucose levels are elevated as in diabetes. Alterations in the permeability occurring as a result of basement membrane changes in diabetes may be an additional explanation for the increased concentration of glucose in saliva.

Belazi et al proposed that the increased permeability of basement membrane in IDDM may lead to an enhanced leakage of serum derived components into whole saliva via gingival crevices.[11] The small molecule of glucose can easily diffuse via the semi permeable basement membrane. They blamed the gingival crevicular fluid to be the culprit for increased glucose level in salivary secretion.

So we can see that the presence of glucose in saliva is multifactorial and not a single mechanism can be blamed.

We have noticed an increased post-prandial salivary glucose level in patients suffering from diabetes mellitus. Hence, it can be concluded that the salivary glucose level can be used as a diagnostic as well as a monitoring tool to assess the glycemic status of a diabetes mellitus patient. But further studies are needed on a larger population and in different geographic areas to establish salivary glucose estimation as a diagnostic as well as a monitoring tool for diabetes mellitus.

CONCLUSION

- Glucose was present in the saliva of both diabetic and nondiabetic subjects.
- Concentration of glucose in the saliva increases with the increase in serum glucose concentration and vice versa.
- A significant correlation was seen between salivary and serum glucose levels in diabetic as well as nondiabetic subjects.

REFERENCES

1. O'Connor PJ, Gregg E, Rush WA, Cherney LM, Stiffman MN, Engelgau MM. Diabetes: how are we diagnosing and initially managing it? Ann Fam Med 2006 Jan;4(1):15-22.
2. Al-Attas SA. Caries experience and selected caries-risk factors among a group of adult diabetics. Saudi Dent J 2008 Sep-Dec; 20(3):129-139.
3. Amer S, Yousuf A, Siddiqui PQR, Junaid A. Salivary glucose concentrations in patients with diabetes mellitus – a minimally invasive technique for monitoring blood glucose levels. Pak J Pharm Sci 2001 Jan;14(1):33-37.
4. Harminka. Spit tests may soon replace many blood tests [news]. Posted 2008 Mar 26, Harminka.
5. Thorstensson H, Falk H, Hugoson A, Olsson J. Some salivary factors in insulin-dependent diabetics. Acta Odontol Scand 1989 Jun;47(3):175-183.
6. Mitsumori M, Yamaguchi M, Kano Y. A new approach to non-invasive measurement of blood glucose using saliva analyzing system. Proceedings of the 20th Annual International

Conference of the IEEE. Mitsumori Engineering in Medicine and Biology Society. Vol. 4. 1998. p. 1767-1770.
7. Carlson AJ, Ryan JG. Glucose in saliva. Am J Physiol 1908;21:301-309.
8. Forbat LN, Collins RE, Maskell GK, Sonksen PH. Glucose concentrations in Parotid fluid and venous blood of patients attending a diabetic clinic. J R Soc Med 1981 Oct;74(10):725-728.
9. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal 2006 Jul 1;11(4):E309-E314.
10. Reuterving CO, Reuterving G, Hägg E, Ericson T. Salivary flow rate and salivary glucose concentration in patients with diabetes mellitus influence of severity of diabetes. Diabete Metab 1987 Jul-Aug;13(4):457-462.
11. Belazi MA, Galli-Tsinopoulou A, Drakoulakos D, Fleva A, Papanayiotou PH. Salivary alterations in insulin-dependent diabetes mellitus. Int J Pediatr Dent 1998 Mar;8(1):29-33.
12. Sreedevi, Shashikanth MC, Shambulingappa P. Comparison of serum glucose and salivary glucose in diabetic patients. J Indian Acad Oral Med Radiol 2008 Jan-Mar;20(1): 9-13.
13. Jurysta C, Bulur N, Oguzhan B, Satman I, Yilmaz TM, Malaisse WJ, Sener A. Salivary glucose concentration and excretion in normal and diabetic subjects. J Biomed Biotechnol 2009;2009:430426.
14. Englander HR, Jeffay AI, Fuller JB, Chauncey HH. Glucose concentrations in blood plasma and parotid saliva of individuals with or without diabetes mellitus. J Dent Res 1963 Sep-Oct;42:1246.
15. López ME, Colloca ME, Páez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. Braz Dent J 2003;14(1):26-31.
16. Qureshi A, Qureshi H, Khan AA. Blood glucose level, salivary pH and oral bacterial count in type 1 diabetic children. Infect Dis J Pak 2007 Apr-Jun;16(2):45-47.