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Estimation & comparison of salivary glucose with blood glucose in diabetic individuals

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***Abstract*---**Aim: Saliva play a diagnostic tool for oral and systematic diseases has multiple advantages over other body fluids especially . The aim of this study was to explore the potential of salivary glucose as a marker in diagnosis and monitoring of diabetes mellitus using glucoseoxidase method, and as a non-invasive method replacing an invasive blood glucose estimation method. Materials and methods: Fasting blood and unstimulated whole saliva were collected from 50

controls, 50 newly diagnosed diabetics, and 50 diabetics under treatment. Blood and salivary glucose were analyzed in the samples by glucose-oxidase method. Results: The mean level of salivary glucose was reported to be 0.53 ± 0.4mg/dl in controls, 1.14 ± 1.55mg/dl in newly diagnosed diabetics, and 1.22 mg/dl ± 1.99 in diabetics under treatment. Conclusion: The mean level of salivary glucose in diabetics was significantly higher than that in non-diabetics. A positive, linear and significant, yet weak correlation between salivary and blood glucose suggests some potential for saliva as a marker in diagnosis and monitoring of diabetes mellitus.

***Keywords*---**Diabetes mellitus; salivary glucose; blood glucose; glucose-oxidase.

Introduction

There is paucity of literature as to correlate salivary glucose with blood glucose among diabetics and non-diabetics. Some reported significantly higher values of glucose in whole saliva in diabetics than in healthy individuals.[1-3] In contrast, other studies of diabetics and healthy individuals indicate that no such relationship exists.[4-6] The international literature with a variety of published data on salivary composition in diabetic patients vary, depending on the type of saliva (whole, parotid, submandibular, unstimulated or stimulated), the different types of diabetes (insulin dependent diabetes mellitus OR non-insulin dependent diabetes mellitus), the differences in the metabolic control of the disease, the ages of the patients, and the duration of diabetes.Investigators have devised and utilized different techniques for the estimation of salivary glucose. These methodological differences have resulted in diverse levels of salivary glucose among diabetics and non-diabetics.

Hence, the purpose of this study was to estimate salivary glucose in diabetics and compare with that in non-diabetic individuals, andto explore potential of salivary glucose as a marker in diagnosis and monitoring of diabetes.

Method

Method and Materials:

Study setting:
This study was conducted in the Department of Oral Pathology & Microbiology, Triveni Dental College & Hospital, Bodri, Bilaspur, Chhattisgarh.

Study population:

Three types of individuals (18 years or above) were considered for participation in this study viz. known diabetics, newly diagnosed diabetics and apparently non-diabetics. All potential participants were explained the need for and procedures of the study. Only those who signed the informed consent were enrolled in the

study. Thus, a total of 150 individuals became study participants distributed as follows:

A. Control group (C) with 50 participants
B. Diabetics under treatment group (D) with 50 participants
C. Newly diagnosed diabetics group (ND) with 50 participants

Each participant's demographic data was obtained and entered in a pre-designed proforma. In diabetics under treatment, details of the type of diabetes, its duration and the treatment were also recorded in the proforma. 2 ml of fasting venous blood and at least 1 ml of fasting unstimulated draining whole saliva samples were collected. R-8C centrifuge (REMI) was used to centrifuge blood samples for 5 min and saliva samples for 10 min at 5250 rpm. Glucose concentration was determined in 10 µl of serum and 500 µl of saliva using a semi-autoanalyzer RA 50 by glucose-oxidase method. Reading was made at 505 nm and the values were recorded.

Statistical analysis:

ANOVA test was used for determining the statistical significance and Pearson's correlation coefficient for determining the correlation of salivary and blood glucose.

Results

The mean age was 57.30 years (±11.74) for control group, 57.82 years (±12.59) for diabetics under treatment group and 56.36 years (±11.299) for newly diagnosed diabetics group and was not significantly different among the 3 groups. The gender distribution was similar among the 3 groups.

Table I represents the statistically non-significant difference of means of salivary glucose among the age groups in the control group (p=0.05) (ANOVA test).

Age (Years)	Mean Salivary Glucose in mg/dl (± SD)
≤35	0.43 (± 0.36)
>35 to ≤45	0.45 (± 0.30)
>45 to ≤55	0.58 (± 0.39)
>55	0.52 (± 0.44)
Whole group	0.53 (± 0.40)

Table I: Salivary Glucose Levels in Control Group

The mean salivary glucose in diabetics under treatment group increased with each advancing age group except for the fourth age group (>55 years) where a substantial decrease in the value was seen which is shown in Table II. However, the differences of means of salivary glucose among the age groups in the diabetics under treatment group was not statistically significant (p=0.05) (ANOVA test).

Age(Years)	Mean Salivary Glucose in mg/dl (± SD)
≤35	0.31 (± 0.00)
>35 to ≤45	0.74 (± 0.51)
>45 to ≤55	2.15 (± 3.11)
>55	1.04 (± 1.69)
Whole group	1.22 (± 1.99)

Table II: Salivary Glucose Level in Diabetics Under Treatment Group

The mean salivary glucose value in the newly diagnosed diabetics group decreased in the second (>35 to ≤45 years) and fourth (>55 years) age group which is represented in Table III. The difference of means of salivary glucose among the age groups was not statistically significant (p=0.05) (ANOVA test).

Age(Years)	Mean Salivary Glucose in mg/dl (± SD)
≤35	0.78 (± 0.61)
>35 to ≤45	0.50 (± 0.21)
>45 to ≤55	1.48 (± 2.05)
>55	1.20 (± 1.55)
Whole group	1.14 (± 1.55)

Table III: Salivary Glucose Level in Newly Diagnosed Diabetics Group

Table IV demonstrates the comparison of salivary glucose levels among the three groups. The difference between the mean level of salivary glucose in non-diabetic individuals (0.53 mg/dl) and that in diabetics under treatment (1.22 mg/dl) is statistically significant (p=0.018). Similarly, the difference between the mean level of salivary glucose in non-diabetic individuals (0.53 mg/dl) and that in newly diagnosed diabetics (1.14 mg/dl) is also statistically significant (p=0.008). The difference between the mean level of salivary glucose in diabetics under treatment (1.22 mg/dl) and that in newly diagnosed diabetics (1.14 mg/dl) is however, statistically not significant (p=0.817).

Group	Mean Salivary Glucose (mg/dl)	Std. Deviation	Number
C	0.53	0.40	50
D	1.22	1.99	50
ND	1.14	1.55	50

Table IV: Comparison of Salivary Glucose Levels among the Three Groups

A statistically non-significant positive correlation was found between salivary glucose and blood glucose in control group (p=0.292, r=0.152) and in diabetics

under treatment group (p=0.149, r=0.207) whereas a statistically non-significant negative correlation was found between salivary glucose and blood glucose in newly diagnosed diabetics group was present (p=0.461, r=-0.107).
But if all the values of salivary glucose in the three groups were pooled together and compared with the pooled serum glucose values, a positive, linear and significant, yet weak correlation existed (r=0.212, p=0.09) (Table V).

Age (Years)	Mean Salivary Glucose in mg/dl (± SD)	Mean Blood Glucose in mg/dl (± SD)
≤35	0.55 (±0.41)	128.80 (±36.10)
>35 to ≤45	0.58 (±0.38)	132.81 (±43.61)
>45 to ≤55	1.30 (±2.06)	141.77 (±55.87)
>55	0.93 (±1.38)	135.52 (±48.96)
Total	0.96 (±1.50)	136.54 (±49.49)

		Salivary Glucose
Blood Glucose	Pearson Correlation	0.212
	p value	0.009
	N	150

Table V: Overall Comparison

Discussion

Diabetes is also known to alter the constitution and flow of saliva. The extent of the alteration and its clinical significance, if any, has been explored by a few researchers. There have been several disparate reports on the relationship between glucose in saliva and blood in healthy individuals and diabetics.
In the present study inconsistent changes in salivary glucose levels across the four age groups were observed in the 3 groups. Several factors influence salivary composition. Whole saliva includes secretions from the gingival crevices. Gingival crevicular fluid (GCF) contains small amounts of glucose but the GCF resting flow in resting flow in healthy individuals is only 0.06 µl and 0.4-1.5 µl from periodontal pockets. Hence, the contribution of glucose by GCF to whole saliva could be considered as insignificant.[8]

The differences in the treatment modalities such as diet, insulin and oral antidiabetics, and the duration of diabetes affect the blood glucose levels in diabetics under treatment group. Similarly, the salivary glucose levels are affected by these two factors. The differences in treatment and duration of diabetes could be additional factors for the inconsistent levels of salivary glucose in diabetics under treatment group.

Mehrotra and Chawla (1968)[9] from their study concluded that salivary sugar presents statistically significant increase with advancing agein diabetics. In non-diabetics, however, increase in the salivary sugar with advancing age was not significant. Nevertheless, this increase in salivary glucose values was not

consistent across the age groups. Similar to age, gender too did not seem to influence salivary glucose levels.
According to the studies carried out by Feller et al (1975)[10], Harrison and Bowen (1987)[11], Reuterving et al (1987)[12] and Borg and Birkhed (1988)[13], salivary flow rate affects glucose values. With increased salivary flow rate, salivary glucose values diminish due to dilution. The salivary flow rate in turn shows diurnal variation. In our study since all the salivary samples were collected at the same time of the day, therefore, salivary flow rate may not be responsible for the variations seen in salivary glucose values.

According to Harrison and Bowen (1987)[11], glucose is a small molecule which easily diffuses through the semi-permeable membranes. This explains presence of glucose in saliva. In diabetics, large amounts of glucose become available to salivary glands.Alterations in the permeability occurring as a result of basement membrane changes in diabetes could be an additional explanation for increased concentrations of glucose in saliva.

Ben-Aryeh et al (1988)[7] suggested that the elevated salivary glucose levels in diabetics could be due to altered microcirculation, autonomic neuropathy, or direct changes in the salivary glands such as damaged basement membranes.
In addition, the increased permeability of basement membrane leads to leakage of serum derived components into whole saliva via gingival crevices (Belazi et al 1998).[14]Thorstensson et al (1989)[15] also reported that it is possible that part of the registered salivary glucose content originates from the gingival fluid.

Between the two diabetic groups, statistically significant difference is not expected as they are affected by the same disorder with similar tissue changes. Longer duration of diabetes may result in irreversible damage to the basement membrane of salivary glands, which may probably explain higher salivary glucose value in diabetics under treatment than in newly diagnosed diabetics.
Studies of glucose concentrations in individual gland saliva also show similar trends. Englander et al (1963)[16] reported that glucose concentration in stimulated parotid saliva varied directly with plasma concentration for each subject, with average glucose concentration in parotid saliva slightly higher than that in non-diabetics.

Significantly higher concentration of glucose in unstimulated parotid saliva in diabetics ($p<0.02$) than controls was found by Sharon et al in 1985.[17]Darwazeh et al (1991)[2] have shown significantly higher concentration of salivary glucose in diabetic patients compared to control group ($p<0.02$), when whole saliva was analyzed.

However, glucose in whole saliva may originate from sources other than salivary glands. Indeed, Sharon et al (1985)[17] made an observation that healthy individuals is only 0.06 µl and 0.4-1.5 µl from periodontal pockets. Hence, the glucose concentration in whole saliva was similar in diabetics and controls.
Reuterving et al (1987)[12] have implied non-existent or individual relationship between serum and salivary glucose concentrations. A threshold mechanism for saliva similar to that of urine at blood glucose concentrations of about 10-15 mmol/l has been suggested.

From the results of this study as well as those from other similar studies, it appears that salivary glucose levels increase with advancing age. The increase, though, is neither significant nor consistent. Biologically, it could be anticipated that salivary glucose levels in diabetics be higher than those in non-diabetics. Results of our study support this expectation.
Correlation analysis of data from individual groups has, thus, failed to establish consistent relationship between salivary glucose and blood glucose. Mehrotra, Chawla and Kumar (1968)[1] also had a similar observation with no set pattern for the non-diabetics.

In all the 3 groups, the correlation is statistically non-significant. It could be presumed that inconsistent salivary glucose levels resulted due to unequal local factors. Presence of some other undetected systemic disease may also have influenced salivary glucose levels in these individuals. Whole saliva is the mixed fluid contents of the mouth and the bacterial flora might use glucose, thereby lowering the concentration.

A critical evaluation of correlation between blood and salivary glucose among the three groups in the present study suggests that a significant positive correlation between them may be observed if each group had a larger sample size.
Darwazeh et al (1991)[2] found a positive correlation between serum glucose and salivary glucose in diabetic patients ($r=0.33$, $p<0.05$). A strong correlation between salivary glucose with blood glucose ($r=0.65$, $p<0.05$) was also reported by Karjalainen et al (1996)[3] and Amer et al (2001)[4] ($r=0.78$).

Tenovuo et al in 1985[5] reported salivary glucose to be four times higher in diabetics than in controls, but in diabetics the relationship between glucose levels in blood and saliva was too weak. Contrary to these studies, Campbell (1965)[6], Sharon et al (1985)[17] and Ben-Aryeh (1988)[7] could not establish correlation between serum glucose and salivary glucose.

The whole saliva is a mixture of secretions and the bacterial flora might use glucose, thereby lowering the concentration.[7] This may explain the weak correlation that exists in our study. In addition, local factors and cross-sectional study design may be responsible for the weak correlation.
Blood glucose levels can change dramatically in short periods of time depending on the type of insulin used and when it is given, when the last meal was eaten, and on the level and duration of physical activity. Transport of glucose from blood to saliva, mostly by diffusion, may not be able to reflect minute changes in blood glucose levels.[3] This probably explains the inter-individual variations among the diabetics.

The main aim of the study was to estimate and compare salivary glucose in non-diabetics and diabetics. We found that the mean level of salivary glucose in diabetics (both in those under treatment and in newly diagnosed) is significantly higher than the mean level of salivary glucose in non-diabetics. We also attempted to establish correlation between salivary glucose and blood glucose. Although weak, a linear positive correlation could be established between salivary glucose and blood glucose.

Supported by the data from other studies, we propose that salivary glucose has a strong potential as a marker in diagnosis and monitoring of diabetes mellitus. However, longitudinal controlled studies with stricter inclusion and exclusion criteria in larger population would be required to convert this potential into clinical practice.

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