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■ Original Article ■

A Comparative Study of the Concentration of Salivary and Blood Glucose in Normal and Diabetic Subjects

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Advantage of saliva analysis are the ease of sample collection and that samples can be collected more frequently with much less stress on the patient. The objective of the present study was to comparatively evaluate the concentrations of saliva and fasting serum glucose in both normal and diabetic subjects. The mean salivary glucose level in diabetic patients was 15.66 ± 17.1 mg/dl and 1.78 ± 1.72 mg/dl ($P = 0.0006$) in the control group. The mean fasting serum glucose level in diabetic patients was 202.12 ± 66.91 mg/dl, while that in the control group was 94.21 ± 14.97 mg/dl ($P < 0.0001$). The 0.95 degree of correlation between salivary and fasting serum glucose could be demonstrated. The concentration of salivary and fasting serum glucose was not significant different betweeen the measurements for male and female. In the oral glucose tolerance test (75g), the glucose concentration in saliva progressively increased during the first 30 minutes of the test and then progressively decreased, reaching at minutes 120 ~ 180 lowest point as like fasting serum glucose concentration. We can conclude that salivary glucose concentration was significantly higher in the diabetic subjects and that there was significant correlation between salivary and fasting serum glucose concentration. Measurement of salivary glucose could be a useful test having good correlation between salivary and fasting serum glucose concentration

Key Words: Saliva glucose, Diabetics, Fasting serum glucose, Oral glucose tolerance test

INTRODUCTION

Saliva is an organic fluid that can indicate local and systemic alterations, such that the components of saliva can be related to the hormonal, immunologic, neurologic, nutritional and metabolic state of the individual (Carlson, 2000). Glucose is a small molecule capable of moving easily through the membranes of blood vessels, passing from the blood plasma to the gingival fluid, via the gingival sulcus, reaching the saliva (Belazi et al., 1998). The increase in blood glucose in the diabetic patient could cause higher levels of salivary glucose with the consequent loss of homeostasis and greater susceptibility to diseases in the oral cavity. The literature, however, shows controversial findings with regard to the comparative values of blood and salivary glucose. There are reports affirming that individuals with elevated levels of blood glucose show oral alterations such as greater incidence of caries (Jones et al., 1992; Pohjamo et al., 1988; Twetman et al., 2002), periodontal disease (Twetman et al., 2002), and candidosis (Karjalainen et al., 1996).

The present research was proposed to conduct a comparative analysis of the concentrations of salivary glucose and fasting serum glucose. Considering the worldwide increase in the incidence of diabetes

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mellitus, research directed towards the measurement of glucose by simple and non-invasive methods, such as collecting saliva, may be useful in the diagnosis of diabetes, thereby improving the quality of life of these individuals. Knowledge of the effects of diabetes on salivary composition and function remains equivocal. Basement membrane permeability of the parotid gland is reported to be higher in diabetes mellitus, and this results in raised percolation of components such as glucose, amylase and protein from blood, thus raising their levels in saliva (Arati et al., 2010; Mealey et al., 2003). Many previous studies have found raised salivary glucose level in diabetes (Arati et al., 2010; Finney et al., 1997; López et al., 2003).

Very few studies have been performed on salivary composition and function in diabetes, particularly in Korea; thus, the data to date are limited. Furthermore, the study results that have been reported are often contradictory in several aspects, and this suggests the need for further investigative studies. The relative inconsistency in the outcomes of various studies may be attributed to variations in the duration of diabetes, the age range of patients, and the metabolic control of diabetes. The potential of saliva to aid in the monitoring of diabetes mellitus was therefore examined in the present study.

The aims of this study were as follow: first, to estimate the concentration of saliva glucose in order to aid in reaching firm conclusion about its alterations in diabetics as compared to healthy non-diabetics; second, to compare and correlate saliva glucose and fasting serum glucose with regard to duration and gender; finally, to assess any significant correlations that may exist between saliva and serum under consideration in the present study.

MATERIAL AND METHODS

Subjects

The group of person included in this study consisted of 50 adults, including 20 males and 30 females between 20 and 76 years of age.

Saliva sample collection

Saliva was collected using a previously published method (Aydin, 2007). Briefly, saliva (5 ml) was collected in fasting subjects, immediately after rinsing their mouths thoroughly with water, then to bend their heads forward and allow saliva to flow into an ice-chilled sterile container bearing the appropriate preservatives. The containers were brought immediately to the laboratory. Collection took 5 min and the salivary flow rate was defined as the volume of saliva secreted per min. Once the saliva was collected, it was centrifuged at 4,000 rpm for 15 min to remove any particulate material. Each supernatant was divided into three aliquots and stored at -20℃ until analysis.

Salivary glucose Assay

Salivary glucose concentration was determined by glucose oxidase end-point method (Arati et al., 2010). Salivary glucose level was determined in saliva samples that were thawed and centrifuged. A pipette was used to transfer 200 μL of the supernatant into previously numbered test tube. Next, 3 mL of enzyme reagent was added to each tube containing saliva. Three standard solutions were prepared, mixing them with the enzyme reagent of the glucose test kit, along with a blank solution composed of only enzyme reagent. The standard solution was used to calculate the level of salivary glucose. The purpose of the blank solution was to zero the spectrophotometer. After preparation of the tubes, they were mixed for a few seconds with a vibrator, in order to homogenize the saliva and enzyme reagent. After mixing well, the samples, standards and blank were incubated in a warm-water bath at 37℃ for 5 min.

The salivary glucose assay were transferred to 1.5 mL cuvettes, and the absorbance was read with a spectrophotometer, at a wavelength 505 nm. After each reading, the sample was discarded and the cuvette was rinsed thrice with distilled water and wiped dry with fine lens paper. Results were calculated and values were expressed as milligrams per deciliter (mg/dl)

Fasting serum glucose were determined using same

method adopted for salivary glucose.

Sensitivity

The lowest concentration that could be distinguished from the zero standard was 15 pg/ml.

Precision

The intra-assay (within-day) variation was determined for two different saliva (S) and two different Fasting serum (FS) samples using the means of two replicates of each. The coefficient of variation (CV) is calculated as: CV = Standard Deviation (SD) / Mean.

Sample	Number	Mean (pg/ml)	SD (pg/ml)	CV (%)
S1	2	24	2.1	8.8
S2	2	21	2.2	10.5
FS1	2	56	4.2	7.5
FS2	2	68	5.6	8.2

The inter-assay (between-days) variation was also determined for two different saliva (S) and two different Fasting serum (FS) samples using the means of several 2 replicates of each.

Sample	Number	Mean (pg/ml)	SD (pg/ml)	CV (%)
S1	3	28	2.7	9.6
S2	3	31	2.5	8.1
FS1	2	74	6.4	8.6
FS2	2	68	6.6	9.7

Linearity

Two saliva (S) and Fasting serum (FS) samples were diluted with distilled water and assayed. (concentrations in pg/ml.)

Sample	Undiluted	1/2	1/4	1/8
S1	28 (100%)	24 (86%)	30 (107%)	32 (114%)
S2	31 (100%)	36 (116%)	42 (134%)	40 (129%)
FS1	74 (100%)	74 (100%)	84 (113%)	82 (110%)
FS2	68 (100%)	72 (106%)	66 (97%)	78 (114%)

Statistical analysis

Statistical analysis was performed using SPSS 14.0 for Windows software. All values are reported as mean ± SD.

RESULTS

As shown in Table 1, in both experimental and control groups, 60% of subject were female and 40% males. The mean age of the experimental group was 53 ± 15 years, and the mean ages of the control group was 37 ± 17 years.

Comparison of glucose concentration in saliva and fasting serum

A first study was conducted in 25 normal subjects, including 10 males and 15 females. The glucose concentration averaged 1.78 ± 1.72 mg/dl (n = 25) in saliva. It did not differ significantly ($P = 0.22$) in male [(2.46 ± 0.75 mg/dl (n = 10)] versus female [1.58 ± 2.41 mg/dl (n = 15)] subjects. A comparable study was then conducted in 25 experimental group, including 10 males

Table 1. Distribution of the groups studied according to sex and age

	Experimental group	Control group
Male	10	10
Female	15	15
Age	53 ± 15	37 ± 17

Table 2. Mean glucose value of salivary and fasting serum in control group

Parameter	Experimental group		*P*- value	Control group		*P*-value
	Male	Female		Male	Female	
Salivary glucose (mg/dL)	9.55 ± 5.24	22.99 ± 23.94	0.21	2.46 ± 0.75	1.58 ± 2.41	0.22
Average	15.66 ± 17.10			1.78 ± 1.72		0.0006
Fasting serum glucose (mg/dL)	183.5 9 ± 52.82	224.35 ± 81.08	0.34	100.34 ± 5.79	92.05 ± 17.93	0.42
Average	202.12 ± 66.91			94.21 ± 14.97		3.1×10^{-8}

and 15 females. The glucose concentration averaged 15.66 ± 17.10 mg/dl (n = 25) in saliva. The glucose concentration failed to differ significantly ($P = 0.21$) in male [9.55 ± 5.24g/dl (n = 10)] and female [22.99 ± 23.94 mg/dl (n = 15)] diabetic patients. As shown in Table 2, the mean salivary glucose concentration for the experimental group was 15.66 ± 17.10 mg/dl and in the control group, 1.78 ± 1.72 mg/dl, a statistically significant difference ($P = 0.0006$). In relation to blood glucose, the experimental group exhibited a mean of 202.12 ± 66.91 mg/dl, while the mean of the control group was 94.21 ± 14.97 mg/dl; also a statistically significant difference ($P < 0.0001$).

In the present study, the lowest sensitivity of saliva glucose was found to be 15 pg/ml. The intra- and inter-assay percentage coefficients of variation for salivary glucose were 8.8 and 10.5, respectively. Determinations in serial dilutions of saliva indicated that the salivary glucose measurement were reliable. Thus, it was verified that the human serum glucose kit could detect saliva glucose quantitatively.

The correlation of blood and saliva glucose concentration

In order to investigate further the relationship between blood and saliva glucose concentration, we compared glucose concentration each person. Fig. 1 illustrates the mean value for saliva glucose concentration and fasting serum glucose each subjects in this study. It documents the increase in salivary glucose with increase fasting serum glucose concentration observed under the same experimental conditions in this study.

The findings, of this study also suggest that a strong positive and significant correlation was found out between the salivary glucose and fasting serum glucose (Fig. 2). The 0.95 degree of correlation between salivary and fasting serum glucose could be demonstrated.

Comparison of oral glucose tolerance test between saliva and fasting serum

In order to investigate further the relationship between blood and saliva glucose concentration, an oral glucose tolerance test (75 g) was conducted in 20 normal subjects (Fig. 3). The glucose concentration in saliva progressively increase during the first 30 minutes of the test, reaching a peak value which averaged 138 ± 33 ($P < 0.01$) of paired basal measurement. Thereafter, the glucose concentration in saliva samples progressively decreased, eventually reaching at minutes 120~180 nadir values representing no more than 70% of paired basal measurement.

DISCUSSION

The use of saliva rather than blood for diagnosis has recently been promoted. Obtaining saliva is advantageous for patients, especially children and diabetic subjects, since the procedure is non-invasive, stress-free and allows

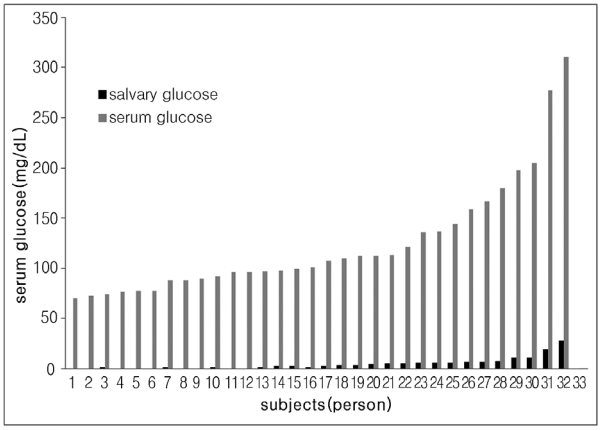

Fig. 1. Comparison of salivary glucose and fasting serum glucose.

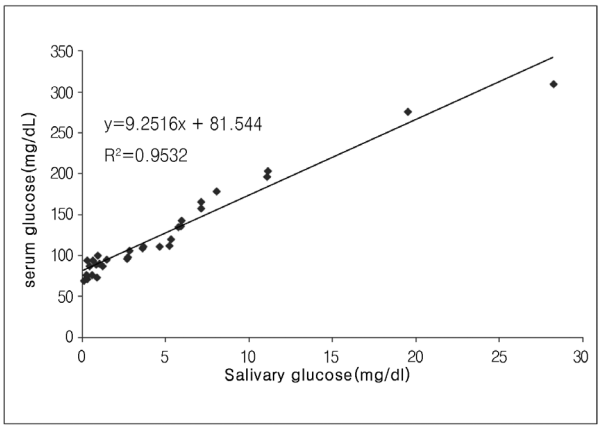

Fig. 2. Correlation between salivary and fasting blood glucose concentration.

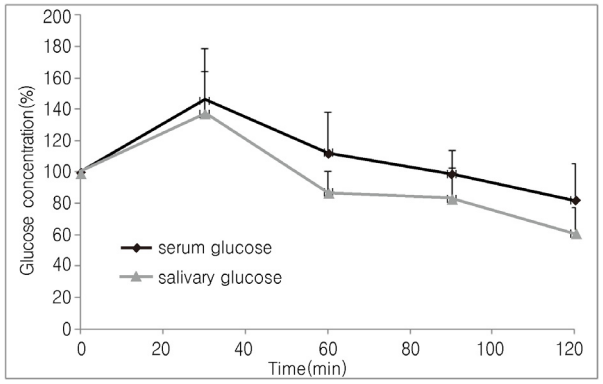

Fig. 3. Time course for the changes in saliva glucose concentration in 20 normal subjects during oral glucose tolerance test.

multiple samplings. Salivary composition in diabetic subjects has been reported in a number of previous studies (Dodds et al., 1997; Belazi et al., 1998; Mata et al., 2004). As the number of diabetes mellitus patients has been increasing recently compared to metabolic syndrome, a simple and non-invasive screening examination should be used universally and the present study contributes to broadening the understanding of the field of blood glucose monitoring. When an easier method than self monitoring of blood glucose is evaluated as reasonable, the diabetes mellitus patients will be free from some burden. Thus, a saliva glucose method and/or modality would be helpful.

The use of saliva as a diagnostic resource has recently prompted studies aimed at determining characteristics of normality. There is a possibility of saliva substituting for blood in some laboratory tests, for example, in determining glycemia in the monitoring of diabetes mellitus, thereby being a non-invasive procedure and allowing multiple sampling (Aydin, 2007; Mata et al., 2004; Di Gioia et al., 2004). As glucose concentration is elevated in diabetics, it is important to compare the levels of salivary and blood glucose in diabetic patients and non-diabetic individuals.

In the present study, we found that the concentration of salivary glucose in diabetic patients was significantly higher than in non-diabetic individuals. This result is in agreement with that obtained by some papers (Aydin, 2007; Ben-Ayred et al., 1988; Carda et al., 2006), who similarly evaluated salivary glucose levels in type 2 diabetic patients and nondiabetics. However, there is divergence with respect to absolute values determined for salivary glucose concentration. It is believed that such differences can be due to differences in methods utilized to determine glucose and in saliva collection.

The correlation between the concentration of blood and of salivary glucose was observed in the present study. This finding corroborates those of Karjalainen (Karjalainen et al., 1996) in studies conducted in diabetic children from whom saliva was stimulated from the parotid glands, showing a correlation between salivary glucose concentration and glycemia. Kjellman (Kjellman, 1970) affirmed the existence of a significant correlation between the concentration of glucose in gingival fluid and glycemia in diabetic patients.

Several papers demonstrated that the level of salivary glucose is augmented only when the concentration of

glucose in blood is elevated (Karjalainen et al., 1996 and Carda et al., 2006). Carda (Carda et al., 2006) observed that only diabetic individuals with fasting glycemia of 180 mg/dl and glycosylated hemoglobin higher than 8%, showed elevated salivary glucose, compared to those patients with poor metabolic control.

The dependency of saliva glucose concentration was further documented by the time course of changes in the former variable during an oral glucose tolerance test, as documented in both control and experimental subjects. During the glucose tolerance test (OGTT) the salivary glucose level increased two-fold within 60 minutes, as observed previously (Andersson et al., 1998; Sreebny et al., 1985). The measurements of saliva glucose concentrations made during such an oral glucose tolerance test led us to observe, in a further set of experiments, that such a concentration decrease at he occasion of successive samplings of stimulated saliva, such a decrease occurred despite unchanged salivary flow. Its pattern was reminiscent of the rapid clearance of exogenous glucose from the saliva of human subjects otherwise observed during the first 6 to 8 minutes, followed by a much slower clearance thereafter (Sreebny et al., 1985; Goulet et al., 1985).

The present study has several potential limitations, such as the small sample size. We were unable to recruit a large number of long duration diabetics and insulin dependant diabetics in our study span. Although the results are not entirely conclusive quantitatively, the study made some novel observations that will unquestionably contribute to providing a platform for further research.

A particularly important issue is the vivid correlations elucidated in this study, and as we cannot propose plausible explanations for them, there is space for further research. The observations derived from this study require more comprehensive evaluation with emphasis on broader representation. We believe that these new perspectives will provide insight in reaching a clear consensus regarding changes in salivary composition and function in diabetes mellitus.

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