Development of Noninvasive Procedure for Monitoring Blood Glucose Levels Using Saliva

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Abstract — This study aim to discuss whether saliva glucose levels (SGL) are applicable to use as an index of diagnosis for diabetic mellitus. We compared the SGL of normal subjects and diabetic subjects. As the results, the data were obtained suggesting that it was preferable to use submandibular and sublingual saliva for estimating blood glucose levels (BGL) as the daily variations in the correlation between BGL and parotid gland SGL in individuals were too big. Also, when SGL of normal subjects and diabetic subjects were compared upon glucose loading, distinct difference are observed between them suggesting that monitoring of SGL can be used for judgment of diabetic mellitus.

Key Words — noninvasive, saliva, blood glucose, diabetic mellitus, OGTT

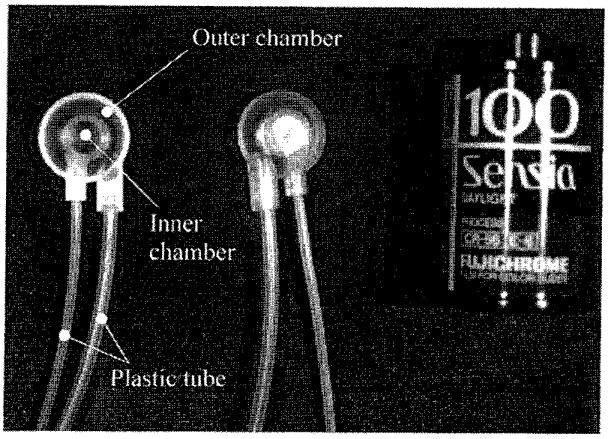
I INTRODUCTION

Self-control of a blood glucose level is a very important measure to keep it in good conditions for diabetic patients during insulin therapy. Since the physical and mental suffers accompanying with blood collecting are occasionally not a small stress for the patients, it is desirable to establish a noninvasive procedure for determining the blood glucose level without taking blood[1].

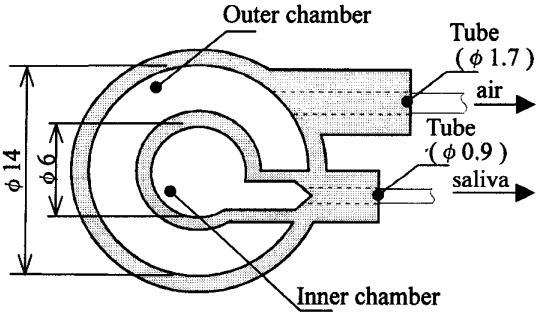
Also, diabetic mellitus, once developed into morbidity, is not only an incurable disease by the current medical technology but is relatively free from specific subjective symptoms. If blood glucose levels (BGL) measuring procedure is developed that can monitor BGL noninvasively, it will ease medical check drastically and enable detection and cure of the disease at an early stage where the disease is called "impaired glucose tolerance (IGT)". However, urinary glucose test now widely used is entangled with relatively high ratio of false results, and therefore it is desired to develop a new procedure[2].

The authors have been attempting to establish a noninvasive procedure to measure the BGL using saliva, one of the most easily obtainable excretes [3],[4]. To achieve this objective, it is necessary to advance in parallel the technological approach regarding development of a salivary analytical procedure[5] and medical approach regarding the correlation between BGL and saliva glucose levels (SGL).

In this paper, we examine the influence of salivary gland to the correlation between BGL and SGL. Submandibular and sublingual saliva and parotid saliva are collected, and 75g oral glucose tolerance test (75gOGTT) is carried out to investigate the time-course changes in BGL and SGL of six normal subjects. Next, submandibular and sublingual saliva of normal subjects and diabetic subjects are collected in order to examine the changes of SGL by diabetic mellitus.



(a) External view



(b) Structure (units in mm)

Fig.1 Parotid saliva collecting devices.
(made by Yoshida Y, Osaka Dental University).

II SALIVARY GLAND

A. Subjects and Method

a. Submandibular and sublingual saliva

The Subjects are six healthy young men, and none of them is affected with periodontitis. The mean (standard deviation, S.D.) age is 24.0 (±4.6) years old, the height is 169.2 (±7.5) centimeters, the weight is 60.5 (±11.3) kilograms, and the fasting blood sugar level (FBS) is 93.1 (±7.9) mg/dl.

OGTT is performed in the subjects who are fasted since the previous evening and blood and saliva are taken simultaneously with 15 minutes interval for 2 hours. Blood collecting is made by finger tip puncture method, and whole saliva mainly excreted from the submandibular and sublingual glands(abbreviate to submandibular and sublingual saliva) is collected under the condition with no stimulus. These samplings are performed for 3 days.

Before collecting saliva, the subjects brushed their teeth clean and well rinsed the oral cavity to remove intraoral residues. After wiping up with cotton rolls for dental use, two cotton rolls are inserted onto the sublingual caruncula to take submandibular and sublingual saliva and left there for about 5 minutes every time. Then, saliva samples are collected by compressing the cotton rolls in a 5 ml syringe (sterilized by γ-ray, Terumo Co., Tokyo) and filtered at 6℃ using a pressurizing ultrafiltration apparatus (Molcut II-LCC, Millipore Co., MA) of which fractional molecular weight is 5000.

SGL is determined using a reagent for the enzymatic method (Glucose C II-Testwako, GOD-POD method, Wako Pure Chemical Industries, Ltd., Tokyo) to measure a glucose concentration. Of the reagent 3 ml is added with 0.2 ml of the saliva sample and incubated at 37 ℃ for 5 minutes after mixing. The optical density at 505 nm is measured by spectrophotometer. A glucose concentration is estimated based on the calibration curve in a range of 0.1～50 mg/dl of which correlation coefficient is 0.99.

The determination of BGL of plasma blood is made by Blood Glucose Auto Analyzer (Antsense, GOD-Electrode, Sankyo Company Ltd., Tokyo).

b.Parotid saliva

The Subjects are six healthy young adults, and none of them is affected with periodontitis. The mean (S.D.) age is 22.0 (±0.9) years old, the height is 168.0 (±7.7) centimeters, the weight is 59.3 (±3.2) kilograms, and the FBS is 100.4 (±5.8) mg/dl.

Parotid saliva is collected using parotid saliva collecting device (Fig.1). This device consists of an inner chamber, an outer chamber, and two plastic tubes. While applying a negative pressure of −10 kPa on the outer camber, the inner chamber is installed on the parotid papilla. To obtain 0.2 ml of saliva that is needed for analysis, only parotid saliva is collected for about 3 minutes each time under stimulation with 0.1 ml of 10% citric acid solution. The parotid saliva is filtered and SGL are analyzed in the similar way as for submandibular and sublingual saliva.

Table 1 Correlation between blood glucose levels and saliva glucose levels in normal subjects.

Subjects	Blood glucose *BG* (mg/dl)	Saliva glucose *SG* (mg/dl)	Time difference t_{bs}*(min)	Sample size *n*	Gradient *a*	Correlation *r*
Submandibular and sublingual saliva						
A	67-168	0.48-2.61	-16± 4	14	22.6	0.89
B	81-191	0.31-1.40	- 3± 4	20	90.3	0.71
C	80-162	0.19-3.82	+ 1± 5	19	13.7	0.65
D	91-155	0.31-1.08	+ 6±11	21	106	0.80
E	93-183	0.15-1.48	-20± 7	12	43.8	0.82
F	92-180	0.27-2.17	+ 3±14	19	10.6	0.66
Mean±SD			- 5±12	17.5±3.6	47.8±41	0.76±0.10
Parotid saliva						
a	97-196	0.03-0.75	+ 8±28	88	30.4	—
b	98-246	0.15-0.66	- 8± 7	104	83.5	0.85
c	97-189	0.17-0.94	+ 4± 1	125	11.8	0.41
d	102-202	0.13-6.10	0± 3	37	5.5	0.48
e	88-204	0.28-0.89	0±10	80	79.5	0.20
f	92-191	0.24-2.84	+ 4±14	74	22.2	0.69
Mean±SD			+ 2±13	84.7±29.7	38.8±34	0.52±0.25

* t_{bs} : The delay of saliva glucose levels based on blood glucose levels.

B. Results

SGL of submandibular and sublingual saliva and parotid saliva are ranging in 0.15～3.82 mg/dl, 0.03～6.10 mg/dl, respectively. Thus, the SGL of parotid saliva expand widely range (Table1). In both saliva, SGL change in pursuit of BGL, and the peaks of BGL and SGL appear with a time lag of t_{bs} =-5±12 min., +2±13min., respectively.

Since the peak times for the two levels are varied among the subjects, the relation between BGL and SGL is examined by linear approximation following coinciding the times of their peak to correct the time-lag(Fig.2). Distinct difference are observed by the kinds of salivary glands in the correlation coefficients. The mean individual correlation coefficient for all subjects are 0.76±0.10, 0.52±0.25, respectively. In particular, there is one case in parotid saliva where no correlation is observed at all.

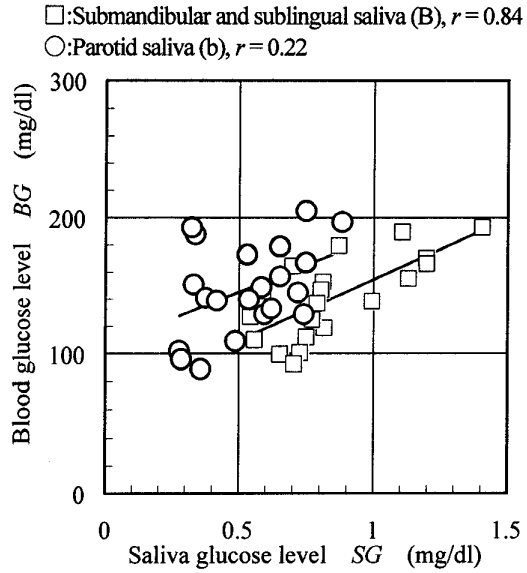


Fig.2 Relationship between BGL and SGL in normal subject for three .consecutive days.

III COMPARISON OF NORMAL SUBJECTS AND DIABETIC SUBJECTS

A. Subjects and Method

The Subjects are eleven diabetic adults(non-insulin-dependent diabetic mellitus, NIDDM), and three subjects of them (c,f,k) are affected with periodontitis. The mean (S.D.) age is 56.3 (±12.2) years old, the incidence years is 9.4 (±8.8) years, the FBS is 153.4 (±42.6) mg/dl, and the HbA_{1c} is 8.7 (±1.6)%(Table2). The measurement methods of BGL and SGL are same as normal subjects. For diabetic subjects, consideration is given not to give physical stress and the interval of sampling of blood and saliva is set as 30 minutes and OGTT is measured only once.

B. Results

In the diabetic subjects, SGL change in pursuit of BGL (Fig.3). BGL and SGL are ranging in 68～364 mg/dl, 1.10～24.85 mg/dl, respectively.

Fig.4 shows comparison of saliva glucose levels between normal subjects and diabetic subjects. The SGL of normal subjects and diabetic subjects are ranging in 0.15～3.82 mg/dl, 1.10～24.85 mg/dl, respectively. In both subjects, distinct difference of about 7-folds at the maximum are observed.

Table 2 Clinical pictures in 11 diabetic subjects (NIDDM) .

Subjects	Sex*1	Age	Incidence years	FBG*2 (mg/dl)	HbA_{1C}*3 (%)	Periodontitis*4
a	F	51	4	138	9.8	-
b	F	70	8	102	6.2	-
c	M	46	7	106	9.4	+
d	F	59	1	139	7.5	-
e	M	63	18	187	7.0	-
f	M	50	10	161	8.9	+
g	M	69	30	139	—	-
h	M	51	1	176	11.5	-
i	F	76	16	215	9.0	-
j	M	35	3	103	—	-
k	M	49	5	221	8.3	+
mean ±SD	—	56.3 ±12.2	9.4 ±8.8	153.4 ±42.6	8.7 ±1.6	—

*1 M : male, F : female, *2 FBG : Fasting blood glucose,

*3 HbA_{1C} : Hemoglobin A_{1C}, normal values are in the range from 4 to 6 %,

*4 + : positive, - : negative

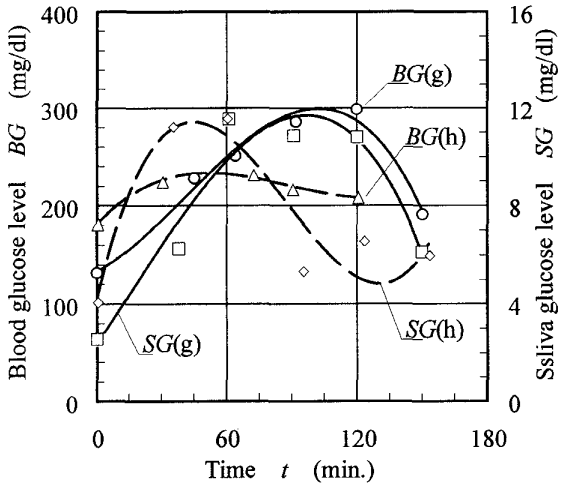


Fig.3 Time-course changes in blood and saliva glucose levels in two diabetic subjects (NIDDM).

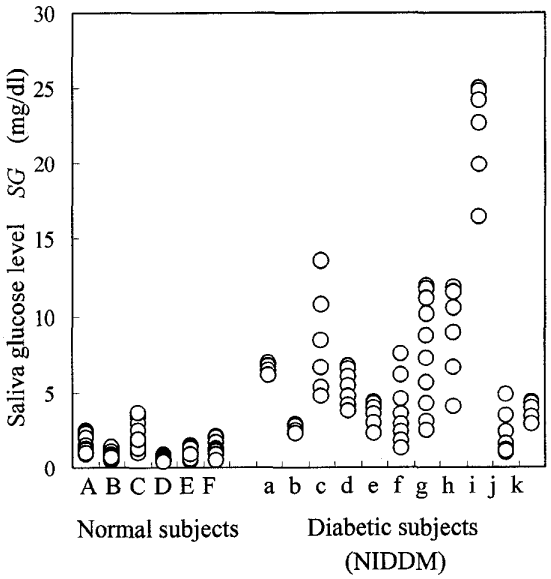


Fig.4 Comparison of saliva glucose levels between normal and diabetic subjects.

IV DISCUSSION

Parotid saliva is easy to collect, but the individual correlation widely changes by days. Thus, it is indicated that the collecting of the submandibular and sublingual saliva is desirable for estimating BGL from the absolute value of self-determined SGL. Furthermore, as submandibular and sublingual saliva collected in this experiment is mixed saliva in a strict sense, by the use of purer submandibular and sublingual saliva collected with a collecting device avoiding mix-up of parotid saliva, further improvements in correlation can be expected.

It is known that there are distinct difference of about 7-folds in the absolute values of SGL of normal subjects and diabetic subjects, and SGL of b and j are under control of BGL by hospitalization are found to show close values to those of normal subjects. From these findings, it is believed that migration of glucose into saliva is affected by diabetic mellitus. Therefore, saying it differently, it is indicated that SGL is applicable to use as an index for diabetic mellitus. In diagnosis, subjects are classified into normal, impaired glucose tolerance and diabetic mellitus. Further, the diabetic mellitus is classified into insulin-dependent diabetic mellitus (IDDM) and NIDDM. Results of detailed investigation in more number of subjects are needed to determine whether or not such classification is possible by this method.

V CONCLUSION

In this study, it is concluded that distinct difference are observed between normal subjects and diabetic subjects suggesting that monitoring of SGL can be used for an index of diabetic mellitus. With further studies on the correlation between diabetic mellitus and SGL, it is believed that not only noninvasive BGL estimation becomes possible, but early detection of diabetic mellitus becomes a reality by the use of this procedure for screening in medical check.

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REFERENCES

[1] WHO Study Group: Prevention of diabetes mellitus, WHO Technical Report Series, 844p., Geneva, 1994.

[2] Ginsberg BH.: An overview of minimally invasive technologies, Clinical Chemistry, vol.38, pp.1596-1600, 1992.

[3] Yamaguchi M., Kimura H., Kano Y., Egusa G., Kita J.: Personal correlation of blood and saliva glucose, The Journal of the Japan Diabetes Society, vol.40, pp.335-340, 1997 [in Japanese].

[4] Yamaguchi M., Kimura H., Mitsumori M., Kano Y.: Experiment on correlation of blood and saliva glucose, Journal of the Society of Life Support Technology, vol.9, pp.3-8, 1997 [in Japanese].

[5] Yamaguchi M., Mitsumori M., Kano Y.: A new approach to noninvasive measurement of blood glucose using saliva, IEEE Engineering in Medicine and Biology Magazine, vol.17, 1998[in press].