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Optical screening of diabetes mellitus using non-invasive Fourier-transform infrared spectroscopy technique for human lip

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ABSTRACT

The Fourier-transform mid-infrared spectroscopy (FTIR) technique has not been used for diabetes diagnosis so far in clinical practice. We attempted to predict non-invasively blood hemoglobin A1c (HbA1c) levels by FTIR to enable evaluation and screening for diabetes. Twenty eight patients from age 20s to 80s, 14 males and 14 females, with and without diabetes, were examined in hospital as a pilot study, and their biochemical data were analyzed with infrared (IR) spectral data of the lip surface by FTIR spectroscopy. Some IR peaks of lip surface had significant correlations with blood glucose and HbA1c levels. Among several peaks in the spectra of lip, peaks at around 1300–1400 cm^{-1} discriminated groups with higher or lower HbA1c levels, suggesting the observation of lip surface advanced glycation end-products with carboxymethyl group. The application of partial least squares (PLS) regression analysis to the correlation between HbA1c levels and lip FTIR spectra resulted in highly significant prediction of HbA1c values for the subjects. Comparison of some IR peaks with predicted HbA1c values served to remove false-negative data effectively. This study with FTIR technique proposed here was effective for screening of diabetes patients with higher HbA1c levels.

1. Introduction

Diagnosis of diabetes mellitus is performed routinely by chemically measuring blood glucose and glycated hemoglobin A1c (HbA1c) or glycated albumin serum levels with a blood sample collected by venous injection. The near-infrared spectroscopy technique may represent a non-invasive method [1,2]. Many proposals for non-invasive measurement of blood glucose level have been published in the last 15 years, including electrical and optical methods using near-IR, mid-IR with Fourier-transform spectroscopy, laser Raman scattering, and various types of fluorescence spectroscopies [3–6].

FTIR spectral analysis reveals differences in several major metabolic components, lipids, proteins, glucose, thiocyanate and carboxylate, and can clearly distinguish healthy and diseased saliva [7]. For example, the lipid ester band at 1735 cm^{-1} was more intense in diabetic saliva, while the bands located at 1400 and 1582 cm^{-1} were less intense [7].

The development of non-invasive measurement of the blood glucose level would be useful for diagnosis of diabetes and screening, and would promote clinical monitoring and permit better management of the disease. It was reported [8] that self-monitoring of blood glucose performed seven or more times per week reduced the lifetime incidence of diabetes-related complications and that periodic testing (1 or 2 times per week) may be cost-effective.

Heise and Marbach [3] used FTIR–ATR (attenuated total reflection) to characterize the outermost layer of human oral mucosa to monitor the blood glucose concentration, and recently Heise and his colleagues also reported [9] that near infrared spectra of different skin regions were recorded for comparison with clinical blood analysis data and further patient information allowing classification into diabetics and non-diabetics.

We have now attempted to evaluate glycation products in the skin layer harboring advanced glycation end products (AGEs), which might be more prevalent in skin cells of diabetics. Glycated -hemoglobin, -albumin, -collagen, and AGEs all were increased in diabetes patients [8,10,11]. We report a new non-invasive screening technique using FTIR–ATR with a novel adaptor for spectroscopy of human lip (lower vermilion portion, not including mucosa) of diabetes patients from ages 20s to 80s, and predict the HbA1c values with removal of false negatives.

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2. Experimental

2.1. Materials and subjects

The measurement of subjects was performed once a week between March 5 and April 2, 2010 at Gifu University Hospital, Gifu, Japan. In total, 28 patients were recruited including 18 with diabetes. The study protocol was approved by the ethics review committee of the Graduate School of Medicine, Gifu University and was in accordance with the principles described in the Declaration of Helsinki. All participating patients gave their written informed consent prior to measurement.

Carboxymethyllysine and carboxymethylarginine were purchased from Funakoshi, Japan. Carboxymethylated dipalmitoylphosphatidylethanolamine (DPPE) was synthesized from standard DPPE (purchased from Sigma, USA) in our laboratory according to the method of Utzmann and Lederer [12], and the product was confirmed by FTIR. Other routine chemicals were obtained commercially. Wet cotton papers (Bioré®) for wiping lips were purchased from Kao Co., Japan. Twenty-eight subjects including diabetes patients and non-diabetes subjects are summarized in Table 1. The value for HbA1c (%) of subjects is estimated as a National Glycohemoglobin Standardization Program (NGSP) value calculated by 0.4% + HbA1c (JDS) [13] measured by HPLC method in the Clinical Laboratory of Gifu University Hospital. The measurement by FTIR was performed in the out-patient physical examination room of Gifu University Hospital before noon (from 9 a.m. to 11 a.m.). Clinical data of the subjects obtained in the hospital were managed by the physician (M.Y.), who is diabetes specialist certified by the Japan Diabetes Society.

Table 1
Baseline characteristics of patients including diabetes and non-diabetes.

	Diabetes group (n = 18)	Non-diabetes group (n = 10)[b]
HbA1c (%)[a]	6.6–10.8 (8.0 ± 1.2)	5.3–6.3 (5.8 ± 0.4)
Blood glucose (mg/dL)	71–285 (159.5 ± 67.8)	83–144 (104.6 ± 21.6)
Age (years)	27–82 (60.4 ± 16.4)	31–80 (66.5 ± 15.4)
Sex (% male)	10 (55.5)	4 (40.0)

Measurements were performed from 2 March to 5 April, 2010 in Department of Clinical Laboratory, Gifu University Hospital.

[a] The value for HbA1c(%) (with mean ± SD) is estimated as an NGSP equivalent value (%) calculated by the formula HbA1c (%) = HbA1c(JDS)(%) + 0.4%, considering the relational expression of HbA1c(JDS)(%) measured by the previous Japanese standard substance and measurement methods as described in the method section [35].

[b] The non-diabetes group includes temporally 1 diabetes patient with lowered HbA1c level (6.2%) by medication, and this grouping is maintained for purpose of HbA1c prediction analysis.

2.2. Measurement of infrared spectra

Fourier-transform infrared spectroscopy with attenuated total reflection adaptor (FTIR–ATR) (TravelIR; SensIR (now Smith Detection Inc.), USA) was used for measurement of IR spectra of lip and skin of human subjects; we refer to this IR system as CISME (Corneum Infrared Spectral analysis for Metabolic Experiments), as previously reported [14], and a specially designed ATR adaptor was made by the financial support of JST (Japan Science and Technology Agency, Tokyo, Japan) and ST Japan Co., (Tokyo, Japan). The lip and face (chin only) skin of the patients was evaluated with this FTIR–ATR device and in this report lip data was presented.

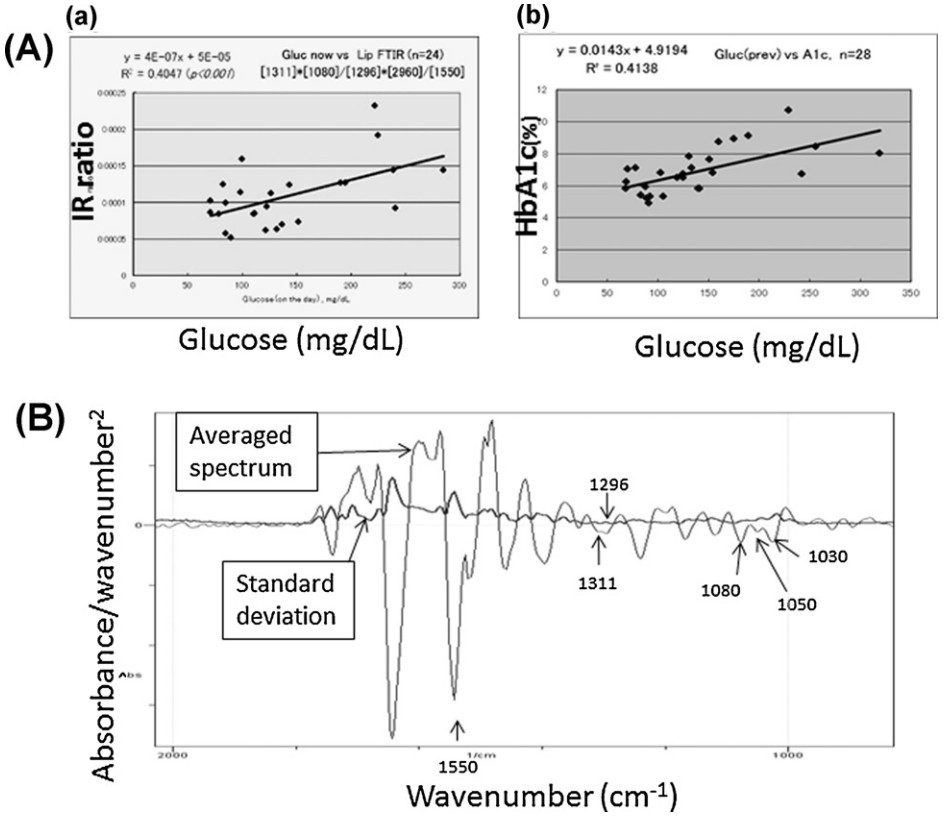

Fig. 1. Correlational analyses between blood data (glucose and HbA1c) and lip skin IR peak ratios of subjects. In (a) of (A), "Gluc now" data vs. lip IR peak ratios is shown (n = 24) after four outliers are removed, where "Gluc now" means the serum glucose level measured on the day of FTIR measurement ("Glucose on the day"); in (b) of (A), HbA1c vs. "Gluc(prev)" is shown, where "Gluc(prev)" means the serum glucose level measured previously (n = 28; nearly 1-month ago of the FTIR measurement). In both (a) and (b), the correlation coefficients (R = 0.64 and 0.63, respectively) are nearly the same. In (B), lip FTIR spectrum in the second derivative form is shown after averaging the spectra of all subjects (n = 28), showing peak positions at 1550, 1311, 1296, 1080, 1050, and 1030 cm^{-1}, and also the standard deviation plots at each wave number are shown. For lip measurement, the IR peak ratio is [1311] × [1080]/[1296] × [2960]/[1550].