Correlation between Molecular Weight of Plasma Albumin Determined with Mass Spectrometry and Glycation Indices in Factory Workers

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Abstract

Objectives: To determine the difference in molecular weights of albumin in factory workers caused by non-enzymatic glycation of plasma albumin using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/MS), and examine the epidemiological validity of this method.

Methods: Forty-eight male workers were tested by random sampling. The difference in molecular weights (ΔM) between the subjects' albumin and human serum albumin was determined by MALDI TOF/MS. Correlations between ΔM vs. fructosamine, fasting plasma glucose (FPG) and HbA1c were investigated.

Results: ΔM showed a significant correlation with all of the tested glycation indices (ΔM vs. fructosamine: $r=0.487$, $p<0.001$) (ΔM vs. FPG: $r=0.482$, $p<0.01$) (ΔM vs. HbA1c: $r=0.397$, $p<0.01$).

Conclusions: Since a significant correlation between ΔM and the glycation indices was found in this investigation, further study with a larger number of subjects is needed for use in clinical applications.

Keywords: albumin, glycation, fructosamine, HbA1c, mass spectrometry

Introduction

Glucose has an aldehyde group with strong reducing power at its end, and this group can combine non-enzymatically with various types of protein while circulating in the blood. Non-enzymatic glycation is used as a blood glucose control index that is not influenced by the latest diet because the concentration of the glycated protein is in proportion to the average blood glucose level in the past. Such proteins as HbA1c (1), glycoalbumin (2) and fructosamine (3) have been clinically applied. In our previous study, we suggested that glycated proteins might also exist in saliva (4).

Lapolla et al. (5) directly determined the molecular weights of albumin (6), globin (7, 8) and IgG (9, 10) in blood with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/MS) method (11, 12). They used the difference in molecular weights caused by combining the proteins with glucose as the result of non-enzymatic glycation, and reported the association between the difference in plasma

protein molecular weights and other glycation indices such as HbA1c and blood glucose. However, what Lapolla et al. examined were samples from healthy subjects and diabetic patients, but no epidemiological survey was performed. In our study, we examined samples taken from a group of workers, and investigated the epidemiological validity of glycated protein determination using MALDI TOF/MS.

Materials and Methods

Subjects

The subjects of the investigation were 48 randomly selected males from a pool of about 500 employees of a metalworking company based in Osaka Prefecture. After overnight fasting, blood samples were collected during the employees' periodical medical check-up, then immediately centrifuged in a cooling centrifuge in the laboratory for 15 minutes at 1200 G and 4°C. The separated plasma samples were kept at -80°C until analysis. The purpose of the investigation was explained to the candidates in advance and their signatures were obtained on an informed consent document.

Chemicals

All reagents were analytical reagent-grade products purchased from Wako Pure Chemical Industries.

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Measurement method

For MALDI measurements, we basically used the method of Lapolla et al. (6), except for the following equipment: an Ultrafree C3-GC membrane (Millipore, cut-off 10,000 Da) for passing 0.5 ml plasma through the membrane in order to eliminate free glucose and salts; a Kubota 1900 centrifugal separator for centrifuging at 7,500 rpm for 30 min; and a Voyager DE-RP mass spectrometer (Applied Biosystems, USA) for MALDI TOF/MS measurements. The MALDI TOF/MS method is based on desorption/ionization by laser irradiation on the protein sample in the presence of suitable matrices, sinapinic acid in this case. The setting conditions of the mass spectrometer were as follows: positive linear mode; accelerating voltage, 20,000 eV; and step voltage, 2,800 eV.

The matrix used was sinapinic acid dissolved in acetonitrile/water (1:1) at a concentration of 5×10^{-3} mol/l. Plasma protein samples were dissolved in water containing 0.1% trifluoroacetic acid to obtain a final albumin concentration of approximately 2×10^{-6} mol/l. We added 5 μ l of that solution to 5 μ l of the matrix solution, and 3 μ l of this mixture was deposited on a

stainless steel sample plate. The droplet was allowed to dry in cool air before being placed into the mass spectrometer. Mass spectra were obtained with an average of ten shots of the laser beam, and the mass values (m/z) were obtained from the Voyager data system. Duplicate MALDI measurements were made for each sample to estimate the reproducibility. The coefficient of variation within a day was always less than 0.1%. Fatty-acid-free human serum albumin (HSA) was used as the molecular weight standard, while bovine serum albumin (BSA) was used as an external calibrant and daily quality control

Table 1 Characteristics of the subjects (n=48)

		means $\pm$ SD
Age	(yr.)	46.0 $\pm$ 8.4
Body mass index	(Kg/m ²)	23.1 $\pm$ 2.5
Fasting plasma glucose	(mg/dl)	109.3 $\pm$ 24.0
Fructosamine	(μ mol/l)	268.8 $\pm$ 40.2
HbA1c	(%)	5.3 $\pm$ 0.9
Δ M	(Da)	154.7 $\pm$ 61.7

Fig. 1 MALDI TOF/MS patterns: (a) the pattern of HSA as baseline, (b) the pattern which showed the minimum Δ M and (c) the pattern which showed the maximum Δ M.

monitor. The ΔM (Da) was obtained using the following equation:

ΔM (Da) = Averaged albumin mass value of sample (m/z) – Averaged albumin mass value of HSA (m/z)

Plasma fructosamine was measured with “Fructosamine test Roche II liquid” and a Hitachi 7150 automatic biochemical analyzer. Fasting plasma glucose (FPG) and HbA1c measured by HPLC and other factors (GOT, GPT, cholesterol, etc.) were analyzed at the Association for Preventive Medicine of Japan. Samples for FPG were collected in test tubes containing NaF.

Statistical analysis

We performed a statistical analysis with SPSS ver. 10.0. The statistically significant level was set to $p < 0.05$, and Pearson's correlation coefficients were used to examine the relationships between the variables.

Results and Discussion

Table 1 outlines the basic characteristics of the 48 subjects

investigated, with 31 persons showing a blood glucose level at 109 mg/dl or lower, 10 persons between 110 and 125 mg/dl and 7 persons at 126 mg/dl or higher. Figure 1 shows typical MALDI TOF/MS spectra for HSA and plasma samples; the mass values reported on those spectra were automatically calculated by the Voyager data system. The peak at 66,327 Da in Fig. 1 (a) indicates the peak of albumin in HSA at one laser shot. The average molecular weight of albumin in HSA at ten shots was 66,325 Da. The difference, ΔM , between the average molecular weight of albumin in HSA and the average molecular weight of albumin in each sample, indicates glycation and/or glyco-oxidation, according to Lapolla et al. (5). They also experimentally showed that incubating BSA in pseudophysiological conditions with glucose at varying concentrations causes a clear increase in BSA molecular weight with respect to incubation time (0–28 days) (13). In the present study, the minimum value of ΔM was 68 Da and the maximum was 338 Da.

Figure 2 shows the correlations between ΔM and fructosamine, ΔM and FPG and ΔM and HbA1c. Clearly, ΔM showed a significant correlation with all of the above glycation

Fig. 2 Correlation between ΔM and fructosamine, FPG and HbA1c (n=48).

Table 2 The correlation matrix between ΔM and the glycation indices (n=48)

	ΔM	FPG	Fructosamine	HbA1c
ΔM (Da)		0.482**	0.487***	0.397**
FPG (mg/dl)			0.746***	0.833***
Fructosamine ($\mu\text{mol/l}$)				0.821***
HbA1c (%)				

FPG, fasting plasma glucose.

: $p < 0.01$ *: $p < 0.001$.

indices. However, no correlation appeared with respect to age, and such clinical laboratory tests as for GOT, GPT and cholesterol. The Pearson's correlation coefficients we obtained were lower than those of Lapolla et al. (6) because Lapolla et al. investigated normal subjects (n=10) and diabetic patients (n=30). In contrast, our subject group (n=48) included few persons with diabetic levels of plasma glucose (n=7).

Table 2 shows the correlation matrix between ΔM and the

glycation indices. Here, HbA1c can be an index of average blood glucose levels for up to three months, while fructosamine can show average levels for up to two weeks. The correlation coefficients between ΔM and the three blood glycation indices were 0.4–0.5, whereas those between the three glycation indices were 0.7–0.8. We presumed that ΔM indicates not only simple glycation of albumin, but also the effect of reactive oxygen species to albumin caused by stress (14, 15).

To the best of our knowledge, the present investigation is the first epidemiological study employing the MALDI TOF/MS method by Lapolla et al. Since the present investigation revealed a significant correlation between ΔM and the glycation indices, this method holds a prospect for clinical application. Moreover, to extend the measurement range of MALDI TOF/MS, a larger amount of information can be gained on the other protein fraction in plasma at the same time. To acquire similar data without MALDI TOF/MS, complex and time consuming procedures would be necessary.

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