Clinical Biochemistry 48 (2015) 397–400

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Clinical

Association of variation range in glycated albumin (GA) with increase but not decrease in plasma glucose: Implication for the mechanism by which GA reflects glycemic excursion

Kunihiko Hashimoto [a], Kazuko Tanikawa [a], Jun Nishikawa [a], Yingchao Chen [a], Toshinobu Suzuki [a], Masafumi Koga [b,*]

[a] Department of Internal Medicine, NTT West Osaka Hospital, Osaka, Japan
[b] Department of Internal Medicine, Kawanishi City Hospital, Hyogo, Japan

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 1 December 2014
Accepted 19 December 2014
Available online 3 January 2015

Keywords:
HbA1c
Glycated albumin
Maillard reaction
Glycemic excursion
Postprandial hyperglycemia

ABSTRACT

Objectives: HbA1c mainly reflects mean plasma glucose (PG), whereas glycated albumin (GA) reflects glycemic excursion in addition to mean PG; the mechanism of the difference between HbA1c and GA is unknown. We hypothesized that a transient increase in PG irreversibly produces stable GA unlike HbA1c. To prove this hypothesis, we investigated diurnal variations in PG, HbA1c, #C fraction (a fraction containing unstable HbA1c and modified hemoglobin on HPLC) and GA in diabetic patients.

Design and Methods: Sixteen diabetic patients with poor glycemic control were enrolled in this study. Blood sampling was performed before and after each meal, before bedtime, and before breakfast on the following day; PG, HbA1c, #C fraction, and GA were measured. The variations of these indicators were compared with those in PG.

Results: HbA1c showed almost no change regardless of diurnal glycemic variation. Variation range in #C fraction significantly correlated with variation range in PG when PG increased ($R = 0.746$, $p < 0.0001$) and decreased ($R = 0.271$, $p = 0.035$). On the other hand, variation range in GA significantly correlated with variation range in PG when PG increased ($R = 0.322$, $p = 0.021$), but not when PG decreased ($R = 0.090$, $p = 0.493$).

Conclusions: We observed that variation range in GA significantly correlated with variation range in PG when PG increased but not when PG decreased for the first time. It is considered that GA reflects glycemic excursion through this phenomenon.

Introduction

HbA1c is widely used as a gold standard of glycemic control indicators [1]. HbA1c is a glycated protein in which glucose is nonenzymatically bound to valine in the β-chain of hemoglobin, and is produced by the two step nonenzymatic reaction called the early Maillard reaction [2]. HbA1c mainly reflects mean plasma glucose (PG); it does not reflect glycemic excursion well. It is considered that this is because a transient increase in PG reversibly produces unstable HbA1c but does not produce stable HbA1c rapidly. HbA1c is observed when hemoglobin is eluted by high-performance liquid chromatography (HPLC). Fractions eluted from unstable HbA1c and modified hemoglobin by the HPLC of Arkray Inc. and Tosoh Corp. are called #C fraction and LA1C+, respectively.

Glycated albumin (GA), another glycemic control indicator, is a glycated protein similar to HbA1c [3]. It is considered that GA reflects glycemic excursion and/or postprandial hyperglycemia in addition to mean PG [3]. Recently, it has been shown that indicators of glycemic excursion determined by continuous glucose monitoring (CGM) have a significant correlation with GA or the GA/HbA1c ratio but not HbA1c [4,5]. Therefore, the GA/HbA1c ratio is high in patients with postprandial hyperglycemia because GA is higher than HbA1c in these patients; when a drug to improve postprandial PG is administered to these patients, the GA/HbA1c ratio decreases, reflecting improvement of postprandial hyperglycemia [6,7]. However, the mechanism of the difference between HbA1c and GA in reflecting PG has been unknown until now, even though they are both glycated proteins.

Regarding the mechanism of the phenomenon in which GA reflects glycemic excursion and/or postprandial hyperglycemia, we hypothesized that it is because the Amadori reaction of GA progresses rapidly, unlike HbA1c. To test this hypothesis, we examined diurnal variations in PG and GA in diabetic patients with poor glycemic control and

* Corresponding author at: Department of Internal Medicine, Kawanishi City Hospital, 5-21-1 Higashi-Uneno, Kawanishi, Hyogo 666-0195, Japan. Fax: +81 72 794 6321.
E-mail address: m-koga@kawanishi-city-hospital.com (M. Koga).

http://dx.doi.org/10.1016/j.clinbiochem.2014.12.021
0009-9120/

investigated the relationship between them. We also investigated diurnal variations in HbA1c and #C fraction and compared with that in PG.

Materials and methods

Subjects studied

Sixteen diabetic patients who were hospitalized at the NTT West Osaka Hospital between May 2013 and November 2013 for glycemic control were enrolled in this study (Table 1). Patients with liver disease, renal disease, anemia, or administration of glucocorticoid, which influence the measurement of HbA1c and/or GA, were excluded. Without change in the treatment of diabetes mellitus immediately after hospitalization, blood sampling was performed before each meal, at 2 h after each meal, before bedtime, and before breakfast on the following day, and PG, HbA1c, #C fraction, and GA were measured and evaluated. The specimens obtained by blood sampling were immediately stored at 4 °C, and measurements were performed within 12 h. This study was approved by the Ethics Committee of the NTT West Osaka Hospital; all patients received an explanation of the aims of this study and gave their written consent.

Laboratory methods

PG was determined by the hexokinase method. HbA1c, expressed as a National Glycohemoglobin Standardization Program (NGSP) value or an International Federation of Clinical Chemistry (IFCC) value (%) [8], and #C fraction were measured by HPLC [9] with an ADAMS-A1c HA-8170 analyzer (Arkray Inc., Kyoto, Japan). Interassay coefficient variations were 0.85% and 0.67%, respectively, as determined in representative blood samples [5.3% (34.4 mmol/mol) and 10.4% (90.2 mmol/mol) of HbA_1c). GA was determined by the enzymatic method using albumin-specific proteinase, ketoamine oxidase, and albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma Co., Tokyo, Japan) [10,11], with a Hitachi 7600 autoanalyzer (Hitachi Instruments Service Co., Tokyo, Japan). Interassay coefficient variations were 1.38% and 1.32%, respectively, as determined in representative serum samples (13.3% and 34.9% of GA). The reference ranges of HbA1c, GA, and #C fraction were between 4.6% and 6.2% (26.7 mmol/mol and 44.2 mmol/mol), between 11.7% and 16.0%, and between 1.5% and 2.0%, respectively.

Statistical analysis

All data are shown as mean ± SD. Single linear univariate regression analysis was employed to assess the association between continuous variables using the StatView computer program (Version 5.0 for Windows, Abacus Concepts, Berkeley, CA). A p value < 0.05 was considered statistically significant.

Table 1
Clinical characteristics of study patients.

Number of patients	16
Men (%)	8 (50)
Type 1 DM/Type 2 DM (n)	2/14
Age (years)	63.8 ± 11.6
BMI (kg/m^2)	27.4 ± 11.6
Duration of diabetes (years)	16.2 ± 13.1
FPG (mg/dL)	145 ± 48
MPG (mg/dL)	174 ± 44
HbA1c (%)	9.0 ± 1.6
HbA1c (mmol/mol)	74.6 ± 17.3
GA (%)	23.9 ± 6.0
#C fraction (%)	2.4 ± 0.3

Results

The characteristics of the study patients included in this study were as follows: eight men and eight women; age: 63.8 ± 11.6 years; BMI: 27.4 ± 11.6 kg/m^2; duration of diabetes: 16.2 ± 13.1 years; two type 1 diabetic patients and 14 type 2 diabetic patients) (Table 1). Fasting PG (145 ± 48 mg/dL), HbA1c (9.0 ± 1.6%; 74.6 ± 17.3 mmol/mol), GA (23.9 ± 6.0%), and #C fraction (2.4 ± 0.3%) in these patients were all high. Before the hospitalization, the treatments of the patients were as follows. Five patients received no medication. Six patients used oral antidiabetic agents. Five patients received insulin therapy, of whom three patients also received oral antidiabetic agents.

Variations range in PG, HbA1c, #C fraction, and GA among blood samples taken at different time points are shown in Fig. 1. PG increased from before meal to after meal and decreased from after meal to before meal. Although PG varied throughout the day, HbA1c showed almost no change. On the other hand, #C fraction varied in a similar way to the variation in PG. Similar to an increase in PG, GA showed an increase from before breakfast to after breakfast, and from before dinner to after dinner. GA showed a decrease from after breakfast to before lunch, from after lunch to before dinner, and from before bedtime to after breakfast; this pattern was generally similar to the variation in PG.

Correlations between variation ranges in HbA1c, #C fraction, and GA and variation range in PG were investigated; no significant correlation was observed between variation range in HbA1c and variation range in PG ($R = 0.012$, $p = 0.896$) (Fig. 2). On the other hand, a significant correlation was observed between variation range in #C fraction and variation range in PG ($R = 0.782$, $p < 0.0001$) and between variation range in GA and variation range in PG ($R = 0.330$, $p < 0.001$).

Furthermore, regarding the correlation between variation range in GA and variation range in PG and the correlation between variation range in #C fraction and variation range in PG, separate analyses were performed for the cases when PG increased from the previous value ($\Delta PG > 0$ mg/dL) and the cases when PG decreased from the previous value ($\Delta PG \leq 0$ mg/dL). Regarding variation range in #C fraction, a significant correlation with variation range in PG was observed in both cases ($R = 0.746$, $p < 0.0001$; $R = 0.271$, $p = 0.035$, respectively); regarding variation range in GA, a significant correlation with variation range in PG was observed only in the case when PG increased ($R = 0.322$, $p = 0.021$), but not when PG decreased ($R = 0.090$, $p = 0.493$) (Fig. 3).

Discussion

In the present study, we examined diurnal variations in glycemic control indicators and PG in diabetic patients with poor glycemic control and analyzed the relationship between each indicator and PG. #C fraction varied in association with glycemic excursion, whereas HbA1c showed almost no change. It is considered that this was because when increased PG decreases, unstable HbA1c is reversibly dissociated to hemoglobin and glucose.

On the other hand, when PG increased, a significant correlation was observed between variation range in GA and variation range in PG, but no such correlation was observed when PG decreased. Because GA is an irreversible Amadori compound, a decrease in GA in association with a decrease in PG was not observed. It is considered that GA reflects glycemic excursion through this phenomenon.

Shima et al. [12] investigated diurnal variation in GA in diabetic patients and reported that GA showed almost no change whereas PG varied. It should be noted that they did not show PG levels; they defined PG before breakfast as 100% and expressed other PG levels relative to it. Unlike the results of the present study, they observed no obvious diurnal variation in GA; this may have been because the patients in their study only had a small glycemic excursion, which resulted in no obvious variation in GA. In contrast, the present study included diabetic patients with poor glycemic control and a marked glycemic excursion.