Relationship between Clinical Markers of Glycemia and Glucose Excursion Evaluated by Continuous Glucose Monitoring (CGM)

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Abstract. In order to evaluate the relationship between clinical markers of glycemia and glucose excursion, we performed 48-hour continuous glucose monitoring (CGM) in 43 diabetic patients. For the clinical markers, HbA1c, glycoalbumin (GA), and 1,5-anhydroglucitol (1,5-AG) were measured, and for the parameters of glucose excursion from CGM, average glucose (AG), standard deviation of glucose (SD), the area under the curve for glucose levels >180 mg/dL (AUC>180), and the difference between the maximum and minimum glucose levels during 48 hours (ΔG48hr) were analyzed. All patients were treated without any changes of the dosages of oral anti-diabetic agents or insulin for at least the previous 6 months with coefficient of variation (CV) of HbA1c less than 4%. In results, while HbA1c did not show any single correlation with AG, SD, AUC>180, or ΔG48hr, both GA and 1,5-AG were significantly related to all these parameters. Furthermore, GA significantly correlated to all CGM parameters, and SD significantly correlated to GA in multiple regression analyses. These results suggest that GA may be a different marker from HbA1c for diabetic complications, because GA, but not HbA1c, may reflect not only short-term average glucose but also fluctuation of glucose.

Key words: Glycoalbumin, Glycated hemoglobin, 1,5-Anhydroglucitol, Continuous glucose monitoring

THE STABLE FRACTION of glycated hemoglobin (HbA1c) is routinely measured in the majority of patients with diabetes around the world, since HbA1c reflects the mean glucose level over the preceding 3 months [1]. HbA1c is not only used to determine whether a patient’s metabolic control has been maintained within the target range, but also to estimate the risk of chronic diabetic complications in each patient. Previous the large-scale prospective studies of diabetic patients have used HbA1c as a marker of glycemic control to evaluate the association of consistent hyperglycemia with the development or progression of chronic diabetic complications [2, 3]. However, recent studies have indicated that postprandial hyperglycemia or fluctuations of the glucose level may be an independent risk factor for macrovascular complications in diabetic patients, which cannot be evaluated by measuring HbA1c alone [4]. Since HbA1c is a marker of the average level of glycemia, it does not reflect acute glucose fluctuations and is poorly correlated with glucose excursions [5]. Therefore, in order to assess the risk of diabetic complications, especially macrovascular complications, it may be necessary to evaluate not only the mean level of glycemic control, but also the extent of glucose excursions such as glucose fluctuations or postprandial elevation of glucose.

To assess daily blood glucose excursions, portable devices for self-monitoring of blood glucose (SMBG) are now widely used by insulin-treated diabetic patients. Although such devices are helpful, the number of measuring times is limited. Recently, a continuous subcutaneous glucose monitoring (CGM) device was developed to evaluate the daily glucose profile in detail. A patient is usually given this device for 3-5 days, and the patient intermittently checks the blood glucose level by using an SMBG device and inputs the
SMBG data into the CGM device [6-9]. By calibration from the SMBG data, the CGM device can provide estimated blood glucose values every 5 min for 3 days. Recently, Nathan et al. evaluated CGM data in 507 subjects, including non diabetic healthy controls and diabetic patients, by 2-day CGM examination every 2 weeks for 12 weeks, and reported that HbA1c was strongly correlated with the estimated average glucose value over the 12 week period ($r^2 = 0.84$, $p<0.0001$) [10]. Although not only the average glucose level, but also the extent of glucose fluctuations or postprandial glycemia, can be evaluated by CGM, they did not analyze these other parameters. We analyzed the standard deviation of glucose levels, the difference between maximum and minimum glucose levels, and postprandial elevation of glucose by 48-hour CGM in diabetic patients, and evaluated the association of these parameters with clinical markers of glycemia such as HbA1c, glycated albumin (GA), and 1,5-anhydroglucitol (1,5-AG).

Materials and Methods

Patients
A total of 43 Japanese patients (26 males and 17 females) with diabetes mellitus (4 with type 1 and 39 with type 2 diabetes) were studied. The patients were recruited from the outpatient clinic of St. Marianna University Hospital between March 2007 and October 2008. In all patients, HbA1c was measured every month at outpatient visits. The inclusion criteria were stable glycemic control (coefficient of variation (CV) of HbA1c < 4 %) without any changes of the dosages of oral anti-diabetic agents or insulin and other drugs. The exclusion criteria included pregnancy, severe medical illnesses, anemia, renal failure (serum creatinine >2.0 mg/dL), overt proteinuria, chronic liver disease, thyroid disease, malignancy, or a history of severe hypoglycemia requiring medical assistance in the previous 6 months. Since measurable range of glucose by CGM was mechanically limited from 40 to 400 mg/dL, the case showing the data out of this range was excluded from the study. All patients gave informed consent and the study was approved by the ethics committee of St. Marianna University Hospital. At least 2 days after admission, the patients were equipped with a first-generation CGM device (Medtronic MiniMed, Northridge, CA, USA), and were monitored for 72 consecutive hours. The characteristics of this device have been described elsewhere [6-9]. During CGM monitoring, patients checked their blood glucose level with an SMBG device (One touch Ultra, Lifescan, Milpitas, CA, USA) at least 4 times per day. Then, they entered the SMBG data and each meal time into the CGM device. After monitoring for 72 hours, the recorded data were downloaded into a personal computer for analysis of the glucose profile and glucose excursion parameters with MiniMed Solutions software. Analysis was limited to the data obtained from the intermediate 48 hours of recording to avoid bias due to insertion and removal of the CGM device or insufficient stability of the monitoring system. On the day of admission, HbA1c was determined by the latex cohesion method (Determiner HbA1c, Kyowa Medex, Tokyo, Japan), serum GA was measured by an enzymatic method using an albumin-specific protease, ketooamine oxidase, and an albumin assay reagent (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan), and 1,5-AG was measured by an enzymatic colorimetric assay kit (Determiner 1,5-AG, Kyowa Medex, Tokyo, Japan).

Assessment of CGM Parameters and Data Analysis
After downloading the recorded data, the following parameters were analyzed from the intermediate 48 hours of data: average glucose (AG), the area under the curve for glucose levels >180 mg/dL (AUC >180), standard deviation (SD) of glucose, and the difference between the maximum and minimum glucose levels during 48 hours ($\Delta G_{48hr}$). Results were presented as means ± SD. Pearson’s univariate regression analysis and stepwise multiple regression analysis were performed to evaluate the relationships between glycemic markers (HbA1c, GA, and 1,5-AG) and the glucose parameters obtained from CGM. Analyses were performed with Stat-View software (Abacus Concepts, Berkeley, CA). Statistical significance was set at $p < 0.05$.

Results
The clinical characteristics, markers of glycemic control, and glycemic parameters obtained from CGM analysis are summarized in Table 1. As shown in Table 2, the univariate correlation of HbA1c with
AG, AUC_{>180}, SD, or ΔG_{48hr} was not significant. However, GA displayed a significant positive correlation with AG ($r = 0.626$, $p < 0.001$), AUC_{>180} ($r = 0.626$, $p < 0.001$), SD ($r = 0.584$, $p < 0.001$), and ΔG_{48hr} ($r = 0.488$, $p < 0.001$). Similarly, 1,5-AG demonstrated a significant negative correlation with AG ($r = -0.453$, $p = 0.004$), AUC_{>180} ($r = -0.413$, $p = 0.009$), SD ($r = -0.472$, $p = 0.002$), and ΔG_{48hr} ($r = -0.457$, $p = 0.004$).

Results of multiple regression analysis employing CGM parameters (AG, AUC_{>180}, SD, and ΔG_{48hr}) as objective variable and using 3 explanatory variables of glycemic markers (HbA_{1c}, GA, and 1,5-AG) were shown in Table 3. HbA_{1c} displayed a significantly negative correlation with AG, AUC_{>180}, and ΔG_{48hr}; 1,5-AG showed a significantly negative correlation with AG and ΔG_{48hr}. However, GA demonstrated a significantly positive correlation with all CGM parameters, and absolute value of standard partial regression coefficient (β) was highest among the glycemic markers (to AG, $β = 0.699$, $p < 0.001$; to AUC_{>180}, $β = 0.720$, $p < 0.001$; to SD, $β = 0.688$, $p < 0.001$; to ΔG_{48hr}, $β = 0.566$, $p = 0.002$).

Results of counter multiple regression analysis employing glycemic markers (HbA_{1c}, GA, and 1,5-AG) as objective variable and using 4 explanatory variables of CGM parameters (AG, AUC_{>180}, SD, and ΔG_{48hr}) was shown in Table 4. While the all CGM parameters were significantly correlated with neither HbA_{1c} nor 1,5-AG, AG and SD showed a significantly positive correlation.
and $\Delta G_{48\text{hr}}$ showed a significantly negative correlation with GA. The absolute value of standard regression coefficient to GA was highest in SD among CGM parameters ($\beta$ in SD = 1.257, $p = 0.011$).

**Discussion**

The present study demonstrated that HbA$_{1c}$ did not show any correlations with CGM parameters such as AG, AUC$_{180\text{hr}}$, SD, or $\Delta G_{48\text{hr}}$, while both GA and 1,5-AG were significantly correlated with all of these CGM parameters in univariate correlation analysis. Only GA was significantly correlated with all CGM parameters in multiple regression analysis, and SD was significantly correlated with GA in counter multiple regression analysis. These results suggest that short-term average glucose levels and glucose excursion cannot be assessed by HbA$_{1c}$ alone, and that GA may reflect not only the average glucose level but also fluctuations of glucose.

To strictly perform multiple regression analysis, each explanatory variable should be independent. However, each glycemic marker (HbA$_{1c}$, GA, and 1,5-AG) correlated mutually, and CGM parameter (AG, AUC$_{180\text{hr}}$, SD, and $\Delta G_{48\text{hr}}$) was also correlated with each other in the present study (data not shown). Actually, HbA$_{1c}$ showed unexpectedly negative correlation to AG, AUC$_{180\text{hr}}$, and $\Delta G_{48\text{hr}}$ as expressed in Table 3, and $\Delta G_{48\text{hr}}$ were also negatively correlated to GA as shown in Table 4, which were different from the results in univariate correlation analysis in Table 2. These discrepancies suggest that the present multiple regression analyses have methodological limitations, and it should be better to accept only an explanatory variable showing highest value of $\beta$ with $p < 0.05$ as at least significantly correlating factor to the objective variable. Considering from this point, GA may be positively correlated to all CGM parameters in Table 3, and SD may be positively correlated to GA in Table 4.

Nathan et al. previously reported a strong correlation of HbA$_{1c}$ with AG from 48-hour CGM at 2-week intervals for 12 weeks, and they proposed a regression equation for estimating the individual average glucose level over two months from the HbA$_{1c}$ value [10]. However, although CV of HbA$_{1c}$ in our patients was less than 4 %, HbA$_{1c}$ was not correlated with AG in univariate regression analysis as shown in Table 2, and AG did not correlate with HbA$_{1c}$ in multiple regression analysis as shown in Table 4. The exact reason why HbA$_{1c}$ did not show a significantly positive correlation with AG is unclear in the present study. One possibility is that the present study was performed in admitting environment and AG obtained from CGM might not reflect ordinary AG level. Another possibility is that daily AG level may actually be fluctuated in or out of clinic even in the patients with stable HbA$_{1c}$ level and hence short-term average glucose level may not be assessed by HbA$_{1c}$ after all. Thus, regarding to the correlation between short term AG and HbA$_{1c}$, further evaluation with larger number of the patients in and out of clinic should be required.

Previously, Dungan et al. also evaluated the relation of CGM parameters to clinical markers of glycemic control in 40 patients with type 1 or 2 diabetes, and demonstrated 3 points: 1) HbA$_{1c}$ was not correlated with AG, similarly to our data, 2) fructosamine (FA) was correlated with both AG and AUC$_{180\text{hr}}$, and 3) 1,5-AG was more robustly related to AUC$_{180\text{hr}}$ than FA [11]. 1,5-AG is now clinically used as a marker of the short-term glycemic state over a couple of days [12-15]. However, persistent glycosuria due to severe hyperglycemia may lead to depletion of the plasma and tissue pool of 1,5-AG, because the renal reabsorption of 1,5-AG by proximal tubular epithelial cells is competitively inhibited by glucose. Thus, the plasma 1,5-AG level cannot precisely reflect the short-term glycemic state in patient with an HbA$_{1c}$ of more than 7 % [16]. As shown in Table 1, the mean HbA$_{1c}$ level of our subjects was higher in the present study (8.0±1.2 %) than in that of Dungan et al. (7.3±0.5%). Thus, 1,5-AG is indeed a useful marker of both average glucose and glucose excursions, but its value may be limited at least in moderately controlled patients. Furthermore, Watanabe et al. previously reported that acarbose, an $\alpha$-glucosidase inhibitor, had an inhibitory effect of intestinal alpha-amylase activity, which plays a role in the digestion of 1,5-AG, so that acarbose therapy decreases the plasma 1,5-AG level due to inhibition of intestinal 1,5-AG digestion and absorption [17]. Accordingly, we should keep this point in mind when assessing glycemic control from 1,5-AG.

FA consists of all glycated plasma proteins, including GA and the unstable fraction of miscellaneous glycated proteins [18]. Specific measurement of GA was initially performed by high-performance liquid chromatography (HPLC) [19]. Then, a new enzymatic method for determining GA was developed, and was confirmed to have sufficient accuracy and to show a
strong correlation with the conventional HPLC method [20]. Since this method can be employed in an auto-analyzer, rapid and multiple determinations can be performed. Therefore, FA has been replaced by GA, and the latter is now widely used as a clinical marker of glycemic control in Japan. Because the turnover of human serum albumin is much more rapid (half-life of 15-20 days) than that of hemoglobin, measurement of GA provides an index of the average glucose level over 2-3 weeks [21]. Interestingly, the present study showed that GA may not only reflect the average glucose level, but also excursions of glucose. Yoshiuchi et al. also recently reported that GA in both type 1 and type 2 diabetic patients was correlated with the maximum blood glucose, but not mean glucose or mean amplitude of glucose excursion (MAGE), obtained from self monitoring of blood glucose in multiple regression analysis, and they discussed that GA could be a useful indicator for postprandial hyperglycemia [22]. Plasma albumin is directly glycated in the blood at four sites of lysine residues, and the glycating reaction is 10 times faster than that for hemoglobin [23], so, it may be advantageous for reflecting rapid changes of the glucose concentration compared with glycated hemoglobin. However, the exact reason why GA is related to daily excursion of glucose remains unclear. Therefore, further basic research and clinical study may be needed to clarify the exact mechanism of GA production.

The present study suggested that GA may be a different marker from HbA1c for diabetic complications, because GA may not only reflect the average glucose level but also glucose fluctuations and postprandial glucose excursions. In fact, Pu et al. have reported that an increase of GA independent of HbA1c is associated with the presence and severity of coronary artery disease in type 2 diabetic patients [24]. However, the present study has several limitations. First, the sample size was not large, and the study population included both type 1 and type 2 diabetic patients. Glycemic fluctuation and excursion in type 1 patients may be different from those in type 2 patients. Second, the patients CGM were performed under admitting situation. Third, the monitoring period was limited to only 72 hours. Thus, in order to confirm the present results, we need further evaluation in large-scaled study by longer period of CGM. It may confirm the usefulness of GA as a complementary marker to HbA1c.

In conclusion, GA was correlated with AG, SD, AUC>180, and ΔG48hr, and SD was significantly correlated to GA. These results may demonstrate that GA reflects not only short term average glucose but also glycemic fluctuation, thus GA may be a different marker from HbA1c for diabetic complications.

References


