To evaluate glycemic control, glycated proteins are often used as glycemic control markers, rather than measuring the actual glucose levels using methods such as self-monitoring of blood glucose (SMBG) and continuous glucose monitoring (CGM). Among the various glycated proteins, glycated hemoglobin (HbA1c) and glycated albumin (GA) are frequently used as glycemic control markers. HbA1c is used as the gold standard index of glycemic control in clinical practice for diabetes treatment \[1\]. It has been reported that these markers are closely associated with the diabetic complications. Since the lifespan of erythrocytes is approximately 120 days, HbA1c reflects the plasma glucose levels over the past few months. The metabolic turnover of albumin is faster than hemoglobin, with a lifespan of approximately 17 to 23 days. Accordingly, GA is used as an index of short-term glycemic control \[2\]. For example, the GA : HbA1c ratio has been suggested to be a better marker of glycemic variability than HbA1c in type 1 diabetes, especially in fulminant type 1 diabetes \[3\]. Importantly, a few past studies have suggested that HbA1c is closely associated with the fasting plasma glucose level, while GA is more closely associated with the postprandial plasma glucose level, compared with the HbA1c level \[4-5\].

Although these glycemic control markers are well correlated with blood glucose levels, HbA1c is influenced by alterations in hemoglobin metabolism and GA is influenced by alterations in albumin metabolism. In clinical practice, conditions such as anemia, chronic renal failure, hypersplenism, chronic liver diseases, hyperthyroidism, hypoalbuminemia, and pregnancy
need to be considered when interpreting HbA1c or GA values [6-13]. However, past studies included the participants who were suffering from those diseases, the selection errors might be caused.

In the present study, we intended to establish a linear regression equation describing the GA value without altered albumin metabolism versus the HbA1c value without altered hemoglobin metabolism to calculate an extrapolated HbA1c (eHbA1c) value for the accurate evaluation of glycemic control. Such an equation would enable quick decisions to be made in clinical practice regarding diabetes treatment based on a given GA value, instead of measuring HbA1c, in patients whose blood control was not stable, changeable within the short-term, or with altered hemoglobin metabolism. Many studies have reported the correlation between HbA1c and GA, but few studies have discussed this correlation in detail. Thus, we investigated the correlation between HbA1c and GA by collecting only data that had not been affected by the turnover of either HbA1c or GA and proposed a novel equation for accurately estimating eHbA1c based on the GA value.

**Materials and Methods**

We retrospectively analyzed the medical charts of patients attending the National Center for Global Health and Medicine (Tokyo, Japan) during 2011 and selected data sets for a total of 2461 occasions were obtained from 731 patients (including non-diabetes patients) whose HbA1c and GA values were simultaneously measured. If these values were measured in the patients on more than one occasion, we selected the data set containing the smallest HbA1c value.

HbA1c was measured using high-performance liquid chromatography (HPLC) (ARKRAY ADAMS-A1C HA-8160; Kyoto, Japan) and was corrected to the National Glycohemoglobin Standardization Program (NGSP) values [14]. GA was measured using an enzymatic method with albumin-specific proteinase, ketoamine oxidase, and an albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma Co., Tokyo, Japan) with the use of an autoanalyzer (Hitachi 770; Hitachi Instruments Service Co., Tokyo, Japan). Each patient was assessed for clinical features such as age, sex, height, body weight, body mass index, blood and urine sample data, history and duration of diabetes mellitus, medications, and complications based on the data contained in the medical records.

We excluded the patients whose previous HbA1c values were missing, or their HbA1c levels were changeable, we selected 550 data sets from the patients as a result of the exclusion. Next, we excluded data sets obtained from patients undergoing hemodialysis and patients with hematological malignancies, pregnancy, chronic liver diseases, hyperthyroidism, steroid treatment, or a blood transfusion during the past 3 months. As a result, 368 data sets remained. Next, we excluded data sets without albumin, hemoglobin, eGFR, or urinary protein measurements and data sets with an eGFR of less than 30 mL/min/1.73 m², a hemoglobin level of less than 10 mg/dL, an albumin level of below 3.0 g/mL, or a urinary protein level of 3+. Finally, we selected 284 data sets (Fig. 1).

This study was approved by the institutional ethical committee of the National Center for Global Health and Medicine and was performed in accordance with the Declaration of Helsinki.

**Statistical analysis**

We performed the statistical analyses using Stata/IC 11. Data on the patient characteristics are shown as the mean ± SE. A total of 284 data sets were used to perform a regression analysis between HbA1c and GA. We have therefore used the bootstrapping method [15] to assess internal validity of the performance of the prediction model. We applied 1,000 resampling procedure with replacement to obtain the bootstrap bias-corrected confidence intervals and the bias estimates (= average of bootstrapped estimates – estimate in the original sample). We also performed stratification according to sex and the change in HbA1c as of the most recent visit (decreased, no change, or increased). Then, for 145 patients in whom body mass index (BMI) data was available, we also stratified the patients according to their BMI (<22 kg/m², ≥22 and <25 kg/m², ≥25 kg/m²) and performed a regression analysis. To evaluate the statistical interaction, we incubated the product interaction terms in the regression models.

**Results**

The 284 individuals whose data were analyzed consisted of 201 men (62.5 ± 0.9 years) and 83 women (65.8 ± 1.6 years), as shown in Table 1. The mean HbA1c was 7.5% ± 0.1% (men) and 7.4% ± 0.2% (women), and the mean GA was 20.9% ± 0.3% (men) and 20.9% ± 0.7% (women). Of the 201 men, 10 individuals had no history.
A novel equation to calculate eHbA1c was established using data from 284 patients. BMI values were available for a total of 145 patients. The mean BMI was 24.9 ± 0.4 kg/m² (108 men) and 23.3 ± 0.6 kg/m² (37 women).

We performed a scatter plot to examine the correlation between HbA1c and GA using data from 284 patients, and established an equation describing the resulting correlation (Fig. 2). Based on all the data points, the resulting equation was as follows: HbA1c = 0.216 × GA + 2.978 [R² = 0.5882, P < 0.001].

The bootstrap bias-corrected 95% confidence intervals (bias estimates) were from 0.193 to 0.238 (0.001) for the slope and from 2.547 of diabetes, 7 patients had type 1 diabetes, 180 patients had type 2 diabetes, and 4 patients had some other type of diabetes. Of the 83 women, 12 individuals had no history of diabetes, 8 patients had type 1 diabetes, and 63 patients had type 2 diabetes. Regarding the medical treatment, the diabetic men were treated using diet therapy (n = 30), oral anti-hypoglycemic agents (n = 143), insulin (n = 52), GLP-1 (n = 4), or combination therapies. The diabetic women were treated using diet therapy (n = 16), oral anti-hypoglycemic agents (n = 57), insulin (n = 22), GLP-1 (n = 2), or combination therapies. Of the 284 patients, BMI values were available for a total of 145 patients. The mean BMI was 24.9 ± 0.4 kg/m² (108 men) and 23.3 ± 0.6 kg/m² (37 women).

We performed a scatter plot to examine the correlation between HbA1c and GA using data from 284 patients, and established an equation describing the resulting correlation (Fig. 2). Based on all the data points, the resulting equation was as follows: HbA1c = 0.216 × GA + 2.978 [R² = 0.5882, P < 0.001].

The bootstrap bias-corrected 95% confidence intervals (bias estimates) were from 0.193 to 0.238 (0.001) for the slope and from 2.547
but no interaction was found in a stratified analysis according to the change in HbA1c (Fig. 3).

Similarly, BMI data for 108 men and 37 women were stratified into 3 groups: <22 kg/m², ≥22 and <25 kg/m², ≥25 kg/m². We performed a scatter plot to examine the correlation between HbA1c and GA. No interaction was found in a stratified analysis according to the change in HbA1c (Fig. 3).
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interaction was found in a stratified analysis according to BMI (Fig. 4).

In addition, we analyzed the correlation by restricting the analysis to the 243 patients with type 2 diabetes. The resulting equation was HbA1c = 0.211 × GA + 3.185 [R² = 0.5307, P < 0.001].

**Discussion**

Our objective for the treatment of diabetes is to prevent the incidence or the progression of the microvascular and macrovascular complications that are specific for diabetes. DCCT (Diabetes Control and Complications Trial), UKPDS (United Kingdom Prospective Diabetes Study), and the Kumamoto Study suggested that better glycemic control was associated with a lower risk of microvascular complications [16-18]. Further, the Funagata Study and the DECODE (Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe) study suggested that blood glucose levels at 2 hours after the 75g OGTT (oral glucose tolerance test) were more strongly associated with the risk of cardiovascular disease (CVD) than fasting glucose levels [19-20]. Recently, the EDIC study (Epidemiology of Diabetes Interventions and Complications), an observational follow-up study performed since the end of the DCCT and in an extended DCCT cohort, was reported [21]. It has been reported that glycemic control markers are closely associated with the diabetic complications [22].

Thus, we investigated the correlation between HbA1c and GA by collecting only data that had not been affected by the turnover of either HbA1c or GA and proposed a novel equation for accurately estimating eHbA1c based on the GA value.

In this study, we analyzed the correlation between HbA1c and GA using clinical data from 284 patients whose HbA1c values were stable, who were not pregnant, and who were free from diseases affecting hemoglobin or albumin metabolism. As a result, the following equation was established: eHbA1c = 0.216 × GA + 2.978.

When the patients were stratified into 3 groups according to the change in HbA1c as of the most recent visit (decreased, no change, or increased), no interaction was found. This result suggests that the glycemic control of the subjects in the present study was sufficiently stable.

The high correlation between HbA1c and GA is well known [23]. Our results are consistent with Tahara’s findings. Tahara examined the correlation using a linear regression analysis among 154 patients with type 2 diabetes. Their regression equation was HbA1c (JDS) = 0.204 × GA + 2.59 [24], which is similar to our equation. Although we enrolled patients with various types of diabetes as well as non-diabetic patients in our study,
most (243 out of 284) of the patients had type 2 diabetes. This may explain the similarity of our findings with those of Tahara. In addition, we analyzed the correlation by restricting the analysis to the 243 patients with type 2 diabetes. The resulting equation was $\text{HbA1c} = 0.211 \times \text{GA} + 3.185$ [$R^2 = 0.5307$, $P < 0.001$], which further supports the similarity of our results with those of Tahara. The differences in the intercepts between the two studies might have arisen because of the difference between the JDS value and the NGSP value. Moreover, by restricting the analysis to the 15 patients with type 1 diabetes, the resulting equation was $\text{HbA1c} = 0.195 \times \text{GA} + 3.173$ [$R^2 = 0.9070$, $P < 0.001$]. Non-diabetic were 22 patients, the correlation between HbA1c and GA was not so strong. Therefore, we could not evaluate its equation model in non-diabetics in this study.

In clinical practice for diabetes treatment, patients suffering from other diseases that could affect the HbA1c data, even if they do not affect the glucose level, are frequently encountered. The HbA1c value can affect the lifespan of erythrocytes, while an aberrant GA value is possible if albumin turnover is changeable. Accordingly, in patients with conditions such as anemia, chronic renal failure, hypersplenism, chronic liver diseases, hyperthyroidism, or hypoalbuminemia, the relationship between HbA1c and GA may be affected. Thus, a careful selection of study participants is important to estimate a reliable correlation between HbA1c and GA.

In patients with hemolytic anemia, because the lifespan of the erythrocytes is shortened, the HbA1c values are lower relative to the plasma glucose level [6]. On the other hand, the HbA1c values are higher in patients with iron deficiency anemia, and false high HbA1c values are observed in iron-deficient states without anemia [7]. During pregnancy, the HbA1c values are higher during the third trimester because of iron deficiency, whereas the GA is not affected. Therefore, GA may be a more suitable marker for monitoring glycemic control during pregnancy [8-9]. In chronic liver diseases, such as chronic hepatitis and liver cirrhosis, hypersplenism lowers the HbA1c values because of the shortened lifespan of the erythrocytes, whereas it raises the GA values because of reduced albumin synthesis and the prolonged half-life of serum albumin [10]. In cases with chronic renal failure, renal anemia lowers the HbA1c values because the lifespan of the erythrocytes is shortened [11]. However, in patients with diabetic nephropathy presenting with marked proteinuria, the GA values are lower because of the increased turnover of albumin metabolism [12]. Hyperthyroidism and steroid treatment, in addition to nephropathy, are known to lower the GA values because of accelerated albumin synthesis. Thyroid hormone is also known to promote albumin metabolism. A study showed that the serum GA level was reduced in patients with thyrotoxicosis, but no apparent change in HbA1c was seen. In addition, GA had significant inverse correlations with the serum free T3 and free T4 levels, as well as a significant positive correlation with the serum TSH level [13]. Additionally, the BMI is known not to affect the HbA1c values, while a negative correlation exists between the BMI and GA. A previous study showed that in obese children, a significant positive correlation was seen between HbA1c and BMI, but a significant negative correlation was seen between GA and BMI [25]. Similarly, in adult diabetic patients, a significant negative correlation between the BMI and the GA level was seen. By contrast, no correlation between the BMI and the HbA1c level was seen [26]. While the reasons for these relations remain unknown, one possible explanation is that obesity increases albumin turnover. Furthermore, chronic inflammatory reactions might also increase albumin turnover. In our study, however, a stratified analysis according to the BMI showed no interaction between these parameters, although the reason for the lack of an interaction was not clear.

Our study had certain limitations. First, we retrospectively selected patients in whom simultaneous HbA1c and GA measurements had been obtained. Thus, a selection bias may exist. Second, as the data were collected from a single hospital and the GA values were not standardized, the present results might not be directly applicable to other hospitals. Although the bootstrap confidence intervals and the bias estimates supported the internal validity of our findings, external validation is needed before applying to other populations. Third, we enrolled patients with various types of diabetes as well as non-diabetic patients in our study. In the future, we are going to investigate the correlation between HbA1c and GA by sorting out the types of diabetes.

In conclusion, we propose an equation for calculating eHbA1c to evaluate the glycemic control of patients with altered hemoglobin metabolism.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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