ORIGINAL ARTICLE

Development of a novel estimation method for hemoglobin A1c using glycated albumin in type 2 diabetes mellitus patients with end-stage renal disease

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Abstract

Aim We developed a novel estimation method for hemoglobin A1c (HbA1c) in type 2 diabetes (T2D) patients with end-stage renal disease (ESRD). This method is based on the glycated albumin (GA) level.

Methods Of the 788 Japanese patients with T2D included in this study, 545 had normal renal function (NRF group) and 243 had ESRD. Oral glucose tolerance tests (OGTTs) were performed in 80 subjects. The variables GA, body mass index (BMI), hemoglobin (Hb), and estimated glomerular filtration rate (eGFR) were significantly associated with the GA-to-HbA1c ratio and were used to determine the estimated HbA1c (eHbA1c). One method of estimating HbA1c involved dividing GA by the GA-to-HbA1c ratio predicted from the estimated regression equation; the estimated HbA1c obtained in this manner was denoted eHbA1c-1.

Results eHbA1c-1 (%) = GA × [4.688 − 18.833 × GA^{-1} − 0.015 × BMI − 0.037 × Hb (− 0.002 × eGFR for patients without ESRD)]$^{-1}$; adjusted R^2 = 0.676 for actual HbA1c. The sensitivity of eHbA1c-1 was better than that of GA for diabetes diagnosis using the 75-g OGTT. There were no differences in the slope of eHbA1c-1 versus GA and the variance of eHbA1c-1 between the ESRD and NRF groups. eHbA1c-1 was not associated with Hb, erythropoiesis-stimulating agent use, or ESRD concomitance.

Conclusions eHbA1c-1 may be a useful parameter for estimating HbA1c in T2D patients with ESRD.

Keywords Type 2 diabetes · End-stage renal disease · Hemoglobin A1c · Glycated albumin

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Introduction

Hemoglobin A1c (HbA1c) is the gold standard marker for glycemic control in patients with diabetes mellitus (DM). However, several studies have suggested that HbA1c levels in end-stage renal disease (ESRD) patients, such as those receiving hemodialysis (HD), tend to be lower for their shorter red blood cell life span and use of erythropoiesis-stimulating agents (ESAs) [1–4]. Other studies have indicated that glycated albumin (GA), which is not affected by the red blood cell life span or ESA administration, could be used as an alternative marker to HbA1c for plasma glucose control in ESRD patients, including those receiving HD [1–3]. However, although GA levels are not influenced by the red blood cell life span or use of ESAs, they may fluctuate under the prolonged albumin (Alb) metabolism associated with common complications experienced by ESRD patients, such as hypothyroidism, decreased body

mass index (BMI), chronic inflammation, and liver dysfunction [5–9]. Thus, there is an ongoing academic debate about whether HbA1c or GA is the more useful glycemic control index in ESRD patients [10–12].

In recent years, several studies have employed the GA-to-HbA1c ratio (GA/HbA1c ratio) to estimate HbA1c levels based on measured GA levels [13–15]. This method is useful for patients in pathophysiological states for which the HbA1c level may not accurately reflect the plasma glucose level. The equation used to predict the HbA1c level in these studies is a linear function in which the GA/HbA1c ratio is assumed to be constant. However, because the GA/HbA1c ratio is 30–40% higher in ESRD patients receiving HD than in DM patients without renal dysfunction [1–3], the conventional predictive equation for HbA1c that uses the measured GA is not suitable for ESRD patients.

To address these issues, we determined the independent factors that contribute to the GA/HbA1c ratio in order to establish a novel regression formula. We then used this formula as the coefficient in novel methods that provided an estimate of HbA1c (eHbA1c) based on the GA level. The purpose of this study was to investigate the utility of one such eHbA1c parameter, eHbA1c-1, derived using one of those novel methods, for type 2 diabetes (T2D) patients with ESRD, including those receiving HD.

Materials and methods

Study design and study subjects

In this cross-sectional study, the medical records of outpatients with and without T2D at the Osafune Clinic, Shin Kashiwa Clinic, Shin Kashiwa Clinic Ootaka No Mori, Kobayashi Medical Clinic, and Innoshima General Hospital were accessed to retrieve background characteristics and clinical data.

First, we developed novel equations for estimating the HbA1c using the GA/HbA1c ratio in T2D patients with normal renal function (NRF) (study design 1). We then investigated the association between the eHbA1c values given by each of two selected novel equations and the plasma glucose level using standard 75-g OGTT data, and assessed the utility of those two eHbA1c equations for diagnosing diabetes mellitus in patients with NRF (study design 2). Finally, we examined the utility of the two eHbA1c parameters (denoted eHbA1c-1 and eHbA1c-2) arising from the two selected novel equations as markers of glycemic control in T2D patients with ESRD (study design 3).

Study design 1

First, we developed novel eHbA1c equations based on the GA level in T2D patients. The clinical data for outpatients with T2D were analyzed and all patients that met the following criteria were considered for inclusion in study design 1: no episodes of ketoacidosis, initial diagnosis of diabetes at > 40 years of age, no demonstrable antibodies to glutamic acid decarboxylase, and no changes in T2D treatment during the 3 months prior to the measurement of HbA1c and GA levels. The GA/HbA1c ratio is influenced by several factors [1–9]. In order to develop a robust eHbA1c parameter, we investigated the independent factors that contribute to the GA/HbA1c ratio in T2D patients without severe anemia or severe hypoalbuminemia. Moreover, T2D patients with the following criteria were excluded from the analysis: diagnosed with DM less than 3 months previously, age < 20 years, signs of infection or treatment for malignancy at the time of specimen collection, and hemoglobin (Hb) level < 8.5 g/dL or serum Alb level < 3.0 g/dL. Subjects in the NRF group had an estimated glomerular filtration rate (eGFR) of ≥ 60 mL/min/1.73 m^2, as calculated from the serum creatinine (Cr) level.

The GA/HbA1c ratio (HbA1c values in derived NGSP units) was calculated using data from T2D patients in the NRF group, and independent factors that contribute to this ratio were investigated. T2D patients with the following characteristics were excluded from this analysis: Hb concentration < 13.5 g/dL in males or < 11.5 g/dL in females, variation in HbA1c ≥ 1.0% (11 mmol/mol) or GA ≥ 2.5% over 3 months, BMI ≥ 30.0 kg/m^2 or BMI < 18.5 kg/m^2, and complication with disorders of the thyroid, liver, and blood, including renal anemia, treatment with ESAs, iron preparations, vitamins, and urinary protein concentration ≥ 0.5 g/gCr. NRF-group subjects with none of these exclusion criteria were included in the final analyses performed to develop equations for predicting HbA1c. A linear regression analysis was performed to identify the variables that influence the GA/HbA1c ratio. A stepwise multivariate linear regression analysis was then carried out to identify the independent variables associated with the GA/HbA1c ratio. A regression formula was derived. Finally, we developed two equations for predicting eHbA1c. In the first, GA was divided by the GA/HbA1c ratio predicted from the regression equation, and the eHbA1c parameter yielded by this equation was denoted eHbA1c-1. In the second equation, HbA1c was predicted from the regression equation, and the eHbA1c parameter yielded by this equation was denoted eHbA1c-2.

Study design 2

We examined the utility of the two eHbA1c equations for diabetes diagnosis. A standard 75-g OGTT was performed

in patients with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), HbA1c ≥ 5.8% (40 mmol/mol), fasting plasma glucose (FPG) ≥ 110 mg/dL, or occasional plasma glucose (PG) ≥ 140 mg/dL, but not in those with a diagnosis of diabetes [16], FPG ≥ 126 mg/dL, eGFR < 60 mL/min/1.73 m^2, age < 20 years, signs of infection or treatment for malignancy, Hb level < 8.5 g/dL, or serum Alb level < 3.0 g/dL at the time of specimen collection. The standard 75-g OGTT was performed after the patient had fasted for at least 10 h. We evaluated PG levels at 0, 30, 60, 90, and 120 min for the 75-g OGTT. We also evaluated C-peptide immunoreactivity (CPR) levels at 0 and 30 min, and calculated ΔCPR by subtracting the CPR at 30 min from the CPR at 0 min, for the 75-g OGTT. In addition, immunoreactive insulin (IRI) levels were evaluated at 0 min for the 75-g OGTT.

Study design 3

We examined the utility of the two eHbA1c equations as markers of glycemic control in T2D patients with ESRD. We investigated whether the average PG (aPG) exhibited any correlation with HbA1c, GA, eHbA1c-1, or eHbA1c-2, and identified the significant independent factors associated with those markers in all T2D patients. In the ESRD group, subjects included those with eGFR < 15 mL/min/1.73 m^2 or those receiving HD. Renal anemia was treated according to Japanese guidelines [17] with ESAs and iron preparations.

Laboratory measures

Blood samples were collected from HD patients before HD therapy, ESRD patients who were not undergoing HD therapy, and NRF-group patients. HbA1c and GA were measured once a month for each subject, concomitant with the measurement of Hb, serum Alb, serum Cr, and urinary protein. aPG levels were measured on three occasions over the three months in the NRF and the ESRD groups. Anemia was defined as Hb < 13.5 g/dL in males and < 11.5 g/dL in females [17]. eGFR was calculated as follows: eGFR = 194 × Cr$^{-1.094}$ × age$^{-0.287}$ (age × 0.739 for females), in accordance with the 2012 Chronic Kidney Disease Guidelines [18]. Homeostasis model assessments (HOMAs) of insulin resistance (HOMA-IR) and HOMA β-cell function (HOMA-β) were carried out based on FPG and IRI levels using the formulae HOMA-IR = FPG (mg/dL) × IRI (μU/mL)/405 and HOMA-β = 360 × IRI (μU/mL)/(FPG (mg/dL) − 63), respectively [19]. PG levels were measured using the HK-G6PD method (DENKA SEIKEN, Tokyo, Japan), HbA1c levels were measured using high-performance liquid chromatography (ARKRAY, Kyoto, Japan), and GA levels were measured using an enzymatic method (Asahikasei Pharma Co., Tokyo, Japan). Plasma IRI levels were measured using

CLEIA (Fujirebio Inc., Tokyo, Japan). Plasma CPR levels were measured using an immunoenzymometric assay (TOSOH Co., Tokyo, Japan).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation values and categorical variables as actual values or percentages. The Shapiro–Wilk test was used to test for Gaussian distributions of continuous variables. The variable urinary protein excretion rate was converted into a natural logarithm (ln). A multiple linear regression analysis was used to investigate the independent factors that contribute to the GA/HbA1c ratio. The multiple correlation coefficient adjusted for the degrees of freedom (adjusted R^2) and the root mean square error (RMSE) were used to investigate the agreement between the calculated eHbA1c values and the actual HbA1c values. The two groups were compared using Student's t test for continuous variables and the chi-square test for frequencies and ordinal variables. Analysis of variance (ANOVA) was used to investigate changes in aPG and HbA1c over three months. Pearson's product-moment correlation coefficient (r) was used to assess correlations between continuous variables. Analysis of covariance (ANCOVA) was applied to examine the congruity of the slope and the variance between two groups. A binomial logistic regression analysis was used to investigate the HbA1c and GA levels in T2D patients in addition to the independent factors that contribute to the eHbA1c. Continuous variables were used in the binomial logistic regression analysis after the values of each variable had been categorized into values above and below its mean. Microsoft Excel® 2010 (Microsoft, Seattle, WA, USA) was used for the RMSE assessment and IBM SPSS® Statistics 19 software (IBM, Armonk, NY, USA) was used for all other analyses. $P < 0.05$ was considered to indicate statistical significance.

Results

Categorization of study subjects

A total of 885 outpatients with type 2 diabetes, IFG, or IGT were enrolled in this study (Fig. 1). The 788 type 2 diabetes patients enrolled in the present study were divided into NRF and ESRD groups (study design 3). Among the 545 NRF-group subjects, 156 selected subjects were examined to develop equations for estimating HbA1c (study design 1). Of the 94 patients that underwent a standard 75-g OGTT, 14 who met the exclusion criteria were eliminated from the analysis. Thus, 80 patients with IFG or IGT were enrolled in the analysis (study design 2).
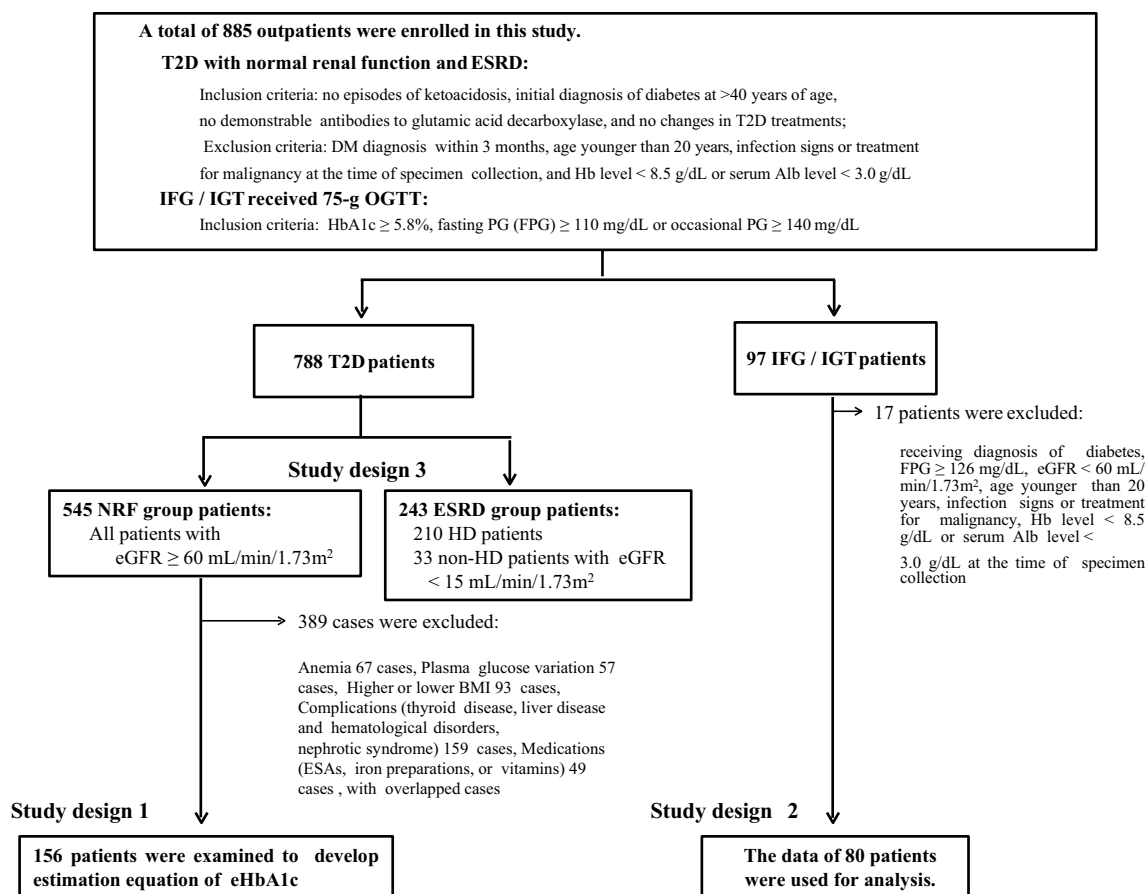
A total of 885 outpatients were enrolled in this study.

T2D with normal renal function and ESRD:

Inclusion criteria: no episodes of ketoacidosis, initial diagnosis of diabetes at >40 years of age,

no demonstrable antibodies to glutamic acid decarboxylase, and no changes in T2D treatments;

Exclusion criteria: DM diagnosis within 3 months, age younger than 20 years, infection signs or treatment

for malignancy at the time of specimen collection, and Hb level < 8.5 g/dL or serum Alb level < 3.0 g/dL

IFG / IGT received 75-g OGTT:

Inclusion criteria: HbA1c ≥ 5.8%, fasting PG (FPG) ≥ 110 mg/dL or occasional PG ≥ 140 mg/dL

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788 T2D patients

97 IFG / IGT patients

17 patients were excluded:

receiving diagnosis of diabetes, FPG ≥ 126 mg/dL, eGFR < 60 mL/min/1.73m², age younger than 20 years, infection signs or treatment for malignancy, Hb level < 8.5 g/dL or serum Alb level <

3.0 g/dL at the time of specimen collection

Study design 3

545 NRF group patients:
All patients with
eGFR ≥ 60 mL/min/1.73m²

243 ESRD group patients:
210 HD patients
33 non-HD patients with eGFR
< 15 mL/min/1.73m²

389 cases were excluded:

Anemia 67 cases, Plasma glucose variation 57 cases, Higher or lower BMI 93 cases, Complications (thyroid disease, liver disease and hematological disorders, nephrotic syndrome) 159 cases, Medications (ESAs, iron preparations, or vitamins) 49 cases, with overlapped cases

Study design 1

156 patients were examined to develop estimation equation of eHbA1c

Study design 2

The data of 80 patients were used for analysis.

Fig. 1 Flow diagram of the present study. A total of 885 outpatients with T2D, IFG, or IGT were enrolled in this study. The 788 T2D patients enrolled in the present study were divided into a NRF group and an ESRD group (study design 3). Among the 545 NRF-group subjects, 156 selected subjects were examined to develop equations for predicting HbA1c (study design 1). Of the 94 patients who underwent a standard 75-g OGTT, 14 who met the exclusion criteria were eliminated from the analysis. Eighty patients with IFG or IGT were ultimately enrolled for analysis (study design 2)

Development of two estimation methods for HbA1c (study design 1)

Of the 545 NRF group subjects, 389 (71.4%) had backgrounds that could possibly influence HbA1c levels and were therefore excluded from the analysis performed to develop the estimation equations. Those subjects had backgrounds that included at least one of the following: anemia (67 cases); plasma glucose variation within the past 3 months (57 cases); high or low BMI (93 cases); thyroid disease, liver disease, hematological disorders, or renal anemia (152 cases); treatment with ESA, iron preparations, or vitamins (49 cases); nephrotic syndrome (7 cases). The selected 156 subjects in the NRF group were examined to identify independent factors associated with the GA/HbA1c ratio. The mean values of HbA1c [% (mmol/mol)] and aPG (mg/dL) over the three consecutive months in the 156 NRF cases were 6.4 ± 0.7 (46 ± 8), 6.5 ± 0.7% (47 ± 8), 6.5 ± 0.7% (48 ± 8), and 6.4 ± 0.7% (46 ± 8) and

127.7 ± 44.2, 133.9 ± 53.1, 129.6 ± 43.8, 123.8 ± 44.5, respectively. Thus, the HbA1c or aPG values were stable before enrollment in the present study (HbA1c, $P = 0.221$; aPG, $P = 0.181$).

The slope of the correlation between the GA/HbA1c ratio and GA was found to be proportional to the inverse of HbA1c, so the GA/HbA1c ratio was nonlinearly correlated with GA. We therefore attempted to identify the GA-based formula that would best predict the GA/HbA1c ratio. We examined inverse ($3.759 - 20.004 \times GA^{-1}$, $R^2 = 0.573$, $P < 0.001$), logarithmic ($-0.932 + 1.235 \times \ln(GA)$, $R^2 = 0.561$, $P < 0.001$), power ($0.624 \times GA^{0.497}$, $R^2 = 0.551$, $P < 0.001$), and exponential ($1.555 \times e^{0.029} \times GA$, $R^2 = 0.517$, $P < 0.001$) models, all employing GA as an independent variable, as possible formulae to predict the GA/HbA1c ratio. We found that the inverse model of GA gave the best fit to the GA/HbA1c ratio among the four models. We thus employed GA^{-1} as an independent variable to estimate the GA/HbA1c ratio in a multiple linear regression

analysis. In addition to GA^{-1}, we also selected age, DM history, degree of proteinuria, serum Alb concentration, BMI, Hb, and eGFR as independent variables. In a stepwise multiple linear regression analysis, age, DM history, degree of proteinuria, and serum Alb concentration were excluded from the regression formula, and GA^{-1}, BMI, Hb, and eGFR were identified as significant independent variables. We then defined eHbA1c-1 as the parameter obtained by dividing GA by the GA/HbA1c ratio predicted from the estimated regression equation, and we defined eHbA1c-2 as the parameter obtained from the estimated regression equation using GA, BMI, Hb, and eGFR.

In the 156 selected T2D patients, eHbA1c-1 and eHbA1c-2 were found to correlate well with the actual HbA1c when equation model 4 and 8 were applied (Table 1), according to the adjusted R^2 and RMSE values. This equation model included the independent variables GA^{-1}, GA, BMI, Hb, and eGFR. It was not possible to evaluate the eGFR in HD patients, and eGFR had a minimal effect on eHbA1c-1 and eHbA1c-2 in the ESRD patients. In fact, the adjusted R^2 values of the equation models with and without eGFR in the ESRD group were 1.000. We therefore eliminated eGFR as an independent variable for ESRD patients. The final equations for eHbA1c-1 and eHbA1c-2 were as follows:

≥ 200 mg/dL and HbA1c $\geq 6.5\%$ (47 mmol/mol). In the 75-g OGTT, eHbA1c-1 $\geq 6.5\%$ significantly predicted peak PG ≥ 200 mg/dL, while both GA $\geq 17.0\%$ [20] ($P = 1.000$) and eHbA1c-2 $\geq 6.5\%$ ($P = 0.374$) failed to predict peak PG ≥ 200 mg/dL. eHbA1c-1 showed superior sensitivity (68%) in the prediction of peak PG ≥ 200 mg/dL and HbA1c $\geq 6.5\%$ (47 mmol/mol) compared with GA (28%) and eHbA1c-2 (40%). The specificity for PG ≥ 200 mg/dL and HbA1c $\geq 6.5\%$ (47 mmol/mol) was similar for all the markers (Table 2).

Correlations of aPG with HbA1c, GA, eHbA1c-1, and eHbA1c-2 (study design 3)

BMI, serum Alb concentration, and serum Hb concentration were significantly lower in the ESRD group ($n = 243$) than in the NRF group ($n = 545$) (Table 3). Although HbA1c values were significantly lower in the ESRD group than in the NRF group, aPG, GA, and eHbA1c-1 values were significantly higher in the ESRD group. The GA/HbA1c ratio was significantly higher (by 30.7%) in the ESRD group than in the NRF group. There were no significant differences between the ESRD and NRF groups in the slopes of the correlations of aPG with HbA1c ($P = 0.065$), GA ($P = 0.137$), eHbA1c-1

$$\text{eHbA1c-1}\,(\%) \,=\, \text{GA} \,\times\, \begin{bmatrix} 4.688 \,-\, 18.833 \,\times\, \text{GA}^{-1} \,-\, 0.015 \,\times\, \text{BMI} \,-\, 0.037 \,\times\, \text{Hb} \\ (-\,0.002 \,\times\, \text{eGFR if the patient does not have ESRD}) \end{bmatrix}^{-1},$$

$\text{eHbA1c-2}(\%) \,=\, 0.099 + 0.218 \times \text{GA} + 0.041 \times \text{BMI} + 0.106$
$\times \text{Hb} \,(+\, 0.005 \times \text{eGFR if the patient does not have ESRD}).$

In the 156 selected T2D patients, HbA1c, eHbA1c-1, and eHbA1c-2 yielded similar regression line slopes (aPG, $P = 0.596$; GA, $P = 0.997$) and variances (aPG, $P = 0.340$; GA, $P = 0.061$) when they were compared with aPG and GA.

Specificity and sensitivity of eHbA1c-1 and eHbA1c-2 for DM diagnosis (study design 2)

Eighty patients (33 males; mean age 59.5 ± 11.8 years old) with IFG or IGT were enrolled in the analysis. eHbA1c-1 and eHbA1c-2 showed significant positive correlations with the PG level at all time points after the 75-g OGTT, and with the peak and mean PG levels after the 75-g OGTT (Table 2). We examined the specificity and sensitivity of eHbA1c-1 and eHbA1c-2 for DM diagnosis based on 75-g OGTT and HbA1c data. Of the 80 patients who received a standard 75-g OGTT, 25 (31.3%) were diagnosed with DM with PG

($P = 0.918$), and eHbA1c-2 ($P = 0.733$) (Fig. 2). However, the variances of HbA1c ($P < 0.001$) and eHbA1c-2 ($P = 0.002$) were significantly lower and the variance of GA ($P < 0.001$) was significantly higher in the ESRD group. Conversely, there was no significant difference between the groups ($P = 0.066$) in the variance of eHbA1c-1 correlated with aPG.

Independent factors that contribute to HbA1c, GA, eHbA1c-1, and eHbA1c-2 (study design 3)

To identify the factors that contribute to HbA1c, GA, eHbA1c-1, and eHbA1c-2, a binomial logistic regression analysis was performed based on the data from all T2D patients (Table 4). The independent variables included aPG, BMI, Alb, presence of anemia, concomitant ESRD, and use of ESAs. aPG and BMI were positive contributing factors to eHbA1c-1, while Hb, use of ESAs, and ESRD concomitance were excluded. In contrast, significant positive contributing factors to eHbA1c-2 were aPG, BMI, and absence of anemia.
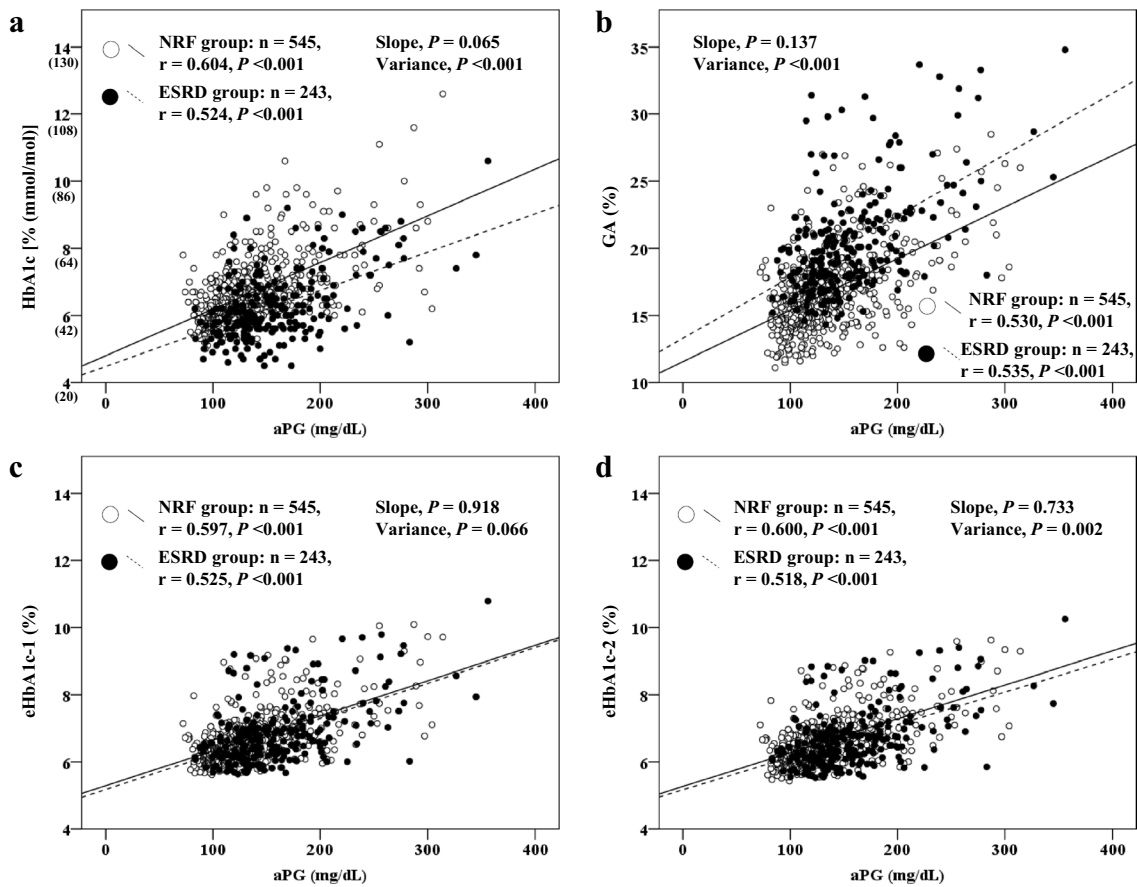
Fig. 2 Relationships of aPG with HbA1c (**a**), GA (**b**), eHbA1c-1 (**c**), and eHbA1c-2 (**d**) in each group. aPG exhibited significantly positive correlations with HbA1c (**a**), GA (**b**), eHbA1c-1 (**c**), and HbA1c 2 (**d**) in the NRF and ESRD groups. No significant differences in slope were noted between the two groups in terms of the relationships of aPG with HbA1c, GA, eHbA1c-1, and eHbA1c-2. However, significant differences were noted between the groups in terms of the vari-ances of HbA1c and eHbA1c-2, which were significantly smaller in the ESRD group than in the NRF group. Moreover, a significant difference was noted between the groups in the variance of GA, which was significantly higher in the ESRD group than in the NRF group. No significant difference in the variance for eHbA1c-1 in relation to aPG was noted between the two groups

Table 1 Adjusted R^2 values and RMSE values for comparisons of four equation models for eHbA1c-1 and eHbA1c-2 with actual HbA1c data for 156 type 2 diabetes patients

Model	eHbA1c-1			eHbA1c-2		
	Equation	Adjusted R^2	RMSE	Equation	Adjusted R^2	RMSE
1	$GA \times (3.759 - 20.004 \times GA^{-1})^{-1}$	0.601[a]	0.454	model 5 $3.106 + 0.209 \times GA$	0.590[a]	0.458
2	$GA \times (4.212 - 19.940 \times GA^{-1} - 0.019 \times BMI)^{-1}$	0.640[a]	0.430	model 6 $1.869 + 0.207 \times GA + 0.053 \times BMI$	0.643[a]	0.428
3	$GA \times (4.512 - 19.146 \times GA^{-1} - 0.015 \times BMI - 0.0032 \times Hb)^{-1}$	0.662[a]	0.415	model 7 $0.733 + 0.215 \times GA + 0.041 \times BMI + 0.049 \times Hb$	0.664[a]	0.415
4	$GA \times (4.688 - 18.833 \times GA^{-1} - 0.015 \times BMI - 0.0037 \times Hb - 0.002 \times eGFR)^{-1}$	0.676[a]	0.415	model 8 $0.099 + 0.218 \times GA + 0.041 \times BMI + 0.106 \times Hb + 0.005 \times eGFR$	0.677[a]	0.406

The adjusted R^2 is the multiple coefficient of determination

The adjusted R^2 and RMSE values indicate that HbA1c is related to eHbA1c-1 or eHbA1c-2

RMSE root mean square error

[a]$P < 0.001$

Table 2 Associations of various characteristics with HbA1c, GA, eHbA1c-1, and eHbA1c-2 in a glucose tolerance test

Number of patients (*n*): 80

Age (years): 59.5 ± 11.8

Gender: male/female (*n*): 33/47

Association of characteristics in 75 g-OGTT		HbA1c		GA		eHbA1c-1		eHbA1c-2	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
HbA1c (% (mmol/mol))	6.3 (45) ± 0.4 (4)	–	–	–	–	–	–	–	–
GA (%)	15.0 ± 1.8	0.141	0.213	–	–	–	–	–	–
eHbA1c-1 (%)	6.4 ± 0.3	0.555	< 0.001	0.410	< 0.001	–	–	–	–
eHbA1c-2 (%)	6.3 ± 0.4	0.508	< 0.001	0.581	< 0.001	0.959	< 0.001	–	–
PG (mg/dL)									
0 min	108.3 ± 11.0	0.388	< 0.001	0.181	0.108	0.515	< 0.001	0.507	< 0.001
30 min	186.3 ± 31.5	0.332	0.003	0.162	0.152	0.312	0.005	0.331	0.003
60 min	213.0 ± 47.5	0.339	0.002	0.073	0.522	0.354	0.001	0.329	0.003
90 min	196.4 ± 55.8	0.294	0.008	0.018	0.877	0.320	0.004	0.284	0.011
120 min	164.1 ± 53.7	0.289	0.009	−0.014	0.899	0.330	0.003	0.298	0.007
Peak	224.5 ± 42.1	0.418	< 0.001	0.149	0.188	0.405	< 0.001	0.400	< 0.001
Mean	173.6 ± 33.4	0.375	0.001	0.065	0.569	0.406	< 0.001	0.379	0.001
Serum CPR (ng/mL)									
0 min	2.0 ± 1.0	0.214	0.056	− 0.526	< 0.001	0.248	0.027	0.131	0.248
ΔCPR	3.0 ± 1.6	− 0.247	0.027	− 0.371	0.001	− 0.349	0.002	− 0.368	0.001
HOMA-IR	2.6 ± 1.7	− 0.003	0.989	− 0.693	< 0.001	0.086	0.677	− 0.060	0.771
HOMA-β (%)	69.6 ± 62.6	− 0.172	0.400	− 0.704	< 0.001	− 0.103	0.617	− 0.245	0.228
BMI (kg/m²)	25.0 ± 5.9	0.313	0.005	− 0.533	< 0.001	0.409	< 0.001	0.265	0.018
Hb (g/dL)	14.2 ± 1.5	0.257	0.022	− 0.285	0.010	0.464	< 0.001	0.388	< 0.001
eGFR (mL/min/1.73 m²)	80.8 ± 14.9	0.142	0.209	− 0.236	0.035	0.063	0.576	− 0.023	0.841

Specificities and sensitivities of HbA1c, GA, eHbA1c-1, and eHbA1c-2 for DM diagnosis	HbA1c ≥ 6.5% (48 mmol/mol)		GA ≥ 17.0%		eHbA1c-1 ≥ 6.5%		eHbA1c-2 ≥ 6.5%	
	%	P^a	%	P^a	%	P^a	%	P^a
Peak PG ≥ 200 mg/dL								
Sensitivity	43.1	0.004	15.5	1.000	44.8	0.038	24.1	0.374
Specificity	90.9		86.4		81.1		86.4	
DM diagnosis[b]								
Sensitivity	100.0	< 0.001	28.0	0.042	68.0	< 0.001	40.0	0.009
Specificity	96.4		90.9		76.4		87.3	

Values are mean ± SD values for continuous variables and numbers for categorical variables

OGTT oral glucose tolerance test, *CPR* C-peptide response, *HOMA-IR* homeostatic model assessment—insulin resistance, *HOMA-β* homeostatic model assessment of beta cells, *ΔCPR* difference between CPR at 30 min and CPR at 0 min, *r* Pearson's correlation coefficient

[a]*P* values were obtained using the chi-square test

[b]Diabetes was diagnosed when peak PG ≥ 200 mg/dL and HbA1c ≥ 6.5% (48 mmol/mol)

Discussion

We propose eHbA1c-1 as a novel parameter for estimating HbA1c values based on GA, BMI, Hb, and eGFR. eHbA1c-1 showed a significant positive correlation with the PG level at all time points, and with the peak and mean PG values during the 75-g OGTT. We found that eHbA1c-1 was closely associated with aPG level but not

with anemia, use of ESAs, serum Alb, or ESRD concomitance in T2D. Our results suggest that eHbA1c-1 may be a useful novel parameter for estimating HbA1c using GA in T2D patients with ESRD. In our study, the GA/HbA1c ratio was significantly increased by 30.7% in the patients in the ESRD group, as previously reported [1–3]. Furthermore, a significant negative correlation was found between eGFR and the GA/HbA1c ratio. In recent years,

Table 3 Clinical characteristics of the subjects

Characteristics	NRF group ($n = 545$)	ESRD group ($n = 243$)	P
Age (years)	62.6 ± 11.2	66.5 ± 11.1	< 0.001
Gender: male/female (n)	272/273	171/72	< 0.001
Duration of diabetes (years)	6.3 ± 6.4	21.6 ± 15.4	< 0.001
Duration of dialysis (years)	–	5.1 ± 4.8	–
BMI (kg/m^2)	24.4 ± 4.3	23.9 ± 4.2	0.122
< 18.5 kg/m^2 (%)	6.1	7.8	0.355
≥ 30.0 kg/m^2 (%)	11.1	8.6	0.602
Serum albumin (g/dL)	4.2 ± 0.3	3.7 ± 0.3	< 0.001
Hemoglobin (g/dL)	13.8 ± 1.6	10.8 ± 0.9	< 0.001
Anemia (%)	11.9	92.0	< 0.001
eGFR (mL/min/1.73 m^2)	83.8 ± 16.6	–	–
Average plasma glucose (mg/dL)	133.6 ± 41.5	156.5 ± 47.0	< 0.001
HbA1c [% (mmol/mol)]	6.7 (49) ± 1.0 (10)	6.3 (45) ± 1.0 (11)	< 0.001
GA (%)	16.7 ± 3.0	20.4 ± 4.0	< 0.001
GA/HbA1c ratio[a]	2.51 ± 0.29	3.28 ± 0.45	< 0.001
eHbA1c-1 (%)	6.7 ± 0.7	6.8 ± 0.9	0.020
eHbA1c-2 (%)	6.6 ± 0.7	6.7 ± 0.9	0.208
Urinary protein (g/gCr)	0.18 ± 0.37	–	–
Medications (%)			
Oral antihyperglycemic agents	76.1	57.2	< 0.001
Insulin therapy	9.0	20.6	< 0.001
Erythropoietin injection	0.0	90.5	< 0.001
Complications and histories (%)			
Liver disease	19.4	9.1	< 0.001
Thyroid disease	6.1	7.0	0.636

Values are mean ± SD values for continuous variables and numbers or percentages for categorical variables

P values for comparisons between two groups were obtained using the t test for parametric continuous variables or the corresponding chi-square test for categorical variables

[a] HbA1c values in derived NGSP units

Table 4 Bivariate logistic regression analysis (stepwise variable selection) of the relationships of HbA1c, GA, eHbA1c-1, and eHbA1c-2 with various characteristics of type 2 diabetes

Variables	HbA1c		GA		eHbA1c-1		eHbA1c-2	
	ORs (95% CIs)	P	ORs (95% CIs)	P	ORs (95% CIs)	P	ORs (95% CIs)	P
aPG	7.40 (5.02–10.89)	< 0.001	5.73 (3.90–8.43)	< 0.001	5.82 (4.09–8.28)	< 0.001	5.82 (4.05–8.35)	< 0.001
BMI	–	–	0.59 (0.40–0.87)	0.007	1.60 (1.13–2.27)	0.008	1.82 (1.29–2.57)	0.001
Alb	–	–	–	–	–	–	–	–
Presence of anemia	0.43 (0.24–0.76)	0.004	–	–	–	–	0.65 (0.43–0.99)	0.044
Presence of ESRD	–	–	9.75 (5.51–17.27)	< 0.001	–	–	–	–
Use of ESAs	0.43 (0.21–0.89)	0.024	–	–	–	–	–	–

Values are odds ratios (ORs) and 95% confidence intervals (CIs)

Values of continuous variables were separated into two groups (0: below the mean value of the variable, 1: above the mean)

several studies have investigated whether HbA1c levels can be estimated from GA levels in patients without ESRD [13–15]. However, eHbA1c-2 and the equations employed in previous studies assume a linear correlation between HbA1c and GA with a constant slope. The formulae used in previous reports [1–3] and eHbA1c-2 are not applicable to ESRD patients, as the GA/HbA1c ratio is large. Instead, eHbA1c-1, which is calculated based on the regression equation for the GA/HbA1c ratio, may be a

useful parameter for monitoring glycemic control in T2D patients with fluctuating GA/HbA1c ratios, such as ESRD patients.

Both Hb and BMI demonstrated significant negative correlations with the GA/HbA1c ratio. Hb was positively associated with HbA1c and negatively associated with GA. Chronic inflammation and malnutrition, which are commonly observed in ESRD patients [21, 22], may enhance Alb catabolism and prolong the survival of erythrocytes. It was shown that both HOMA-β and BMI are negatively correlated with the GA/HbA1c ratio and positively correlated with HbA1c [22, 23]. In another study, GA values were found to be elevated in patients with a low BMI and a low HOMA-IR [5]. Thus, patients with a high BMI may be mistakenly diagnosed as nondiabetic based on their GA values [20]. eHbA1c-1 may be a useful marker not only for T2D patients with ESRD but also in other patients in whom the HbA1c level may not exactly reflect the actual PG level, such as those suffering from anemia, obesity, or malnutrition.

Previous reports [1–3] showed that there was no difference in blood glucose levels and GA values between diabetic patients with ESRD and those without nephropathy. However, in our study, significantly higher GA variance was observed in the ESRD group, and concomitant ESRD was a significant independent contributing factor to higher GA levels. One reason for this discrepancy between studies may be that T2D patients were classified by Cr value in previous reports but by eGFR value in this study. However, further studies are necessary to clarify this discrepancy.

There are some limitations of this study. Firstly, the blood samples from patients who were not receiving HD were obtained in an outpatient clinic and were collected before dialysis sessions from HD patients. A similar approach to sample collection was also used in previous studies examining the interrelationships between PG, HbA1c, and GA in T2D patients [1–3, 24]. Further investigation based on data provided by continuous glucose monitoring is needed to resolve this limitation [25]. Moreover, the utility of eHbA1c for diagnosing diabetes mellitus in T2D patients with ESRD has not been demonstrated. Further clinical studies are required to address this issue. Secondly, the number of patients enrolled in this study was rather small. An analysis using a large database of diabetic patients encompassing a variety of races and ethnicities is needed to develop more accurate eHbA1c-1 equation models. Finally, eHbA1c-1, which is calculated using a GA-based equation, may reflect faster changes in plasma glucose levels than in HbA1c.

In conclusion, we have proposed a novel method of estimating HbA1c from GA using a GA/HbA1c ratio regression formula. The resulting parameter, eHbA1c-1, may be useful for diagnosing DM and for monitoring glycemic control in T2D patients with ESRD. Further studies are need to confirm the usefulness of this parameter for ESRD patients with diabetes.

Compliance with ethical standards

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