Estimation of the Hemoglobin Glycation Rate Constant

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Abstract

Aim: In a previous study, a method of obtaining mean erythrocyte age (M_{RBC}) from HbA1c and average plasma glucose (AG) was proposed. However, the true value of the hemoglobin glycation constant (k_g dL/mg/day), required for this model has yet to be well characterized. Another study also proposed a method of deriving M_{RBC} from erythrocyte creatine (EC). Utilizing these formulae, this study aimed to determine a more accurate estimate of k_g.

Methods: 107 subjects including 31 patients with hemolytic anemia and 76 subjects without anemia were included in this study. EC and HbA1c data were analyzed, and M_{RBC} using HbA1c, AG and the newly-derived constant, k_g were compared to M_{RBC} using traditional ^{51}Cr in three patients whose data were taken from previous case studies.

Results: A value of 7.0×10^{-6} dL/mg/day was determined for k_g. M_{RBC} using HbA1c, AG and k_g were found to no be significantly different (paired t-test, $p = 0.45$) to M_{RBC} using traditional ^{51}Cr.

Conclusions: k_g enables the estimation of M_{RBC} from HbA1c and AG.

keywords:
erythrocyte creatine, hemoglobin glycation, erythrocyte lifespan, hemolysis

1 Introduction

HbA1c is widely used as both an indicator of glycemic control, as well as a diagnostic index, for diabetes in clinical settings [1,2]. Although HbA1c is generally indicative of recent glycemic control over the past 1–2 months, it is known to show reduced correlation to glycemic control status in the presence of diseases which result in a shortened erythrocyte lifespan such as hemolytic anemia [3].

We have recently proposed a simple method to obtain mean erythrocyte age (M_{RBC}) from HbA1c and average glucose (AG) [4]:

$$M_{RBC} \simeq \frac{HbA1c}{(1-\frac{2}{3}HbA1c)k_g \mathrm{AG}} \tag{1}$$

where k_g is the rate constant of the glycation reaction. This formula provides meaningful information for the diagnosis of anemia. We estimated k_g to be 6–10$\times 10^{-6}$ dL/mg/day based on past literature [4]. However, a more accurately estimated value of k_g would provide more useful information.

The relationship between M_{RBC} and erythrocyte creatine (EC) was previously established based on a model [5] as following:

$$M_{RBC} = -22.84 \log_e EC + 65.83 \tag{2}$$

This study aimed to determine the accurate value of k_g from EC-derived M_{RBC} and HbA1c.

2 Materials and Methods

2.1 Participants

107 subjects including 31 patients with hemolytic anemia and 76 subjects without anemia were included in this study. All samples were prepared and analyzed in accordance with the protocols approved by the institutional committees at Kumamoto University and other collaborating institutions.

Patients with hemolytic anemia were recruited from 115 patients who were older than 20 years old and required laboratory tests including complete blood counts and reticulocyte counts (ret) for clinical reasons. Those who were suspected of having diabetes mellitus (DM) based on history, a low 1,5-AG value (male, <14.9 μg/mL; female, <12.4 μg/mL), or had comorbid liver or renal diseases, were excluded, as liver and renal diseases affect HbA1c and glycated albumin (GA). EC, HbA1c, GA, haptoglobin, and other biochemical screening items were measured using the existing plasma samples from these patients. Use of existing plasma samples from anemic patients without written consent was approved by the institutional review board.

Participants without anemia were recruited from medical examination checkup recipients at Takagi Hospital. Those who had anemia, DM, liver disease, renal disease or who were pregnant were excluded to avoid confounding effects on HbA1c or GA value. We provided the healthy volunteers with detailed information about the study, and all participants without anemia provided written informed consent to participate.

2.2 Data interpretation

HbA1c expressed in International Federation of Clinical Chemistry (IFCC) units (iA1c) was used for calculations in this study. While the National Glycohemoglobin Standardization Program (NGSP) is used to express HbA1c in many clinical research and medical care settings, NGSP is measured by an old standardized method and at the time of conception, high performance liquid chromatography (HPLC) was not able to distinguish true HbA1c from other products. HPLC technology later advanced, however the derived HbA1c value is adjusted to NGSP in the interest of consistency. IFCC provides a strict definition of iA1c as hemoglobin with a glycated valine in the β-chain. Thus, iA1c value is preferred value for estimation of erythrocyte glycation.

To acquire iA1c from HbA1c expressed in NSGP unit, we used the following equation [6]:

$$HbA1c_{\mathrm{NGSP}}\,(\%) = 0.0915\times \mathrm{iA1c}\,(\mathrm{mmol/mol}) + 2.153\% \tag{3}$$

$$\iff \mathrm{iA1c(mmol/mol)} = 10.93\times HbA1c_{\mathrm{NGSP}}\,(\%) - 23.53 \tag{4}$$

M_{RBC} was acquired from EC by the aforementioned equation (2).

An AG value of 100 mg/dL was substituted for blood glucose values derived using CGM. This number was based on the average AG of non-diabetic participants and the previously reported findings from a study which showed the median AG in healthy subjects to be reported to be 101.0 (96.3 – 106.0) mg/dL [7] and another ADAG (A1c-Derived Average Glucose) study which found that the AG of the non-diabetic group of their study was similarly 100mg/dL [8,9].

M_{RBC} was also determined using ^{51}Cr half-life. As the reference range for ^{51}Cr half-life was described as 28–30 days [10], 30±5 days [11],

and 26–40 days [12], M_{RBC} was calculated by multiplying ^{51}Cr half-life and 2.14 (=60/28), 60 days being the normal value for M_{RBC}.

2.3 Data analysis

EC and M_{RBC} data were analyzed using a spreadsheet software, Excel® 365 (Microsoft Corporation, Redmond, WA, USA).

2.4 Estimation of k_g

The following two methods were used to estimate k_g. The slope method – the following equation (5) derived from equation (1) shows that the slope of the line connecting a point and the origin is k_gAG.

$$\frac{\text{iA1c}}{1000 - \frac{2}{3}\text{iA1c}} = k_g\text{AG} \times M_{RBC} \tag{5}$$

Estimating the slope of the regression line through the origin by the least square model:

$$\frac{\sum_i^n x_i y_i}{\sum_i^n x_i^2} \tag{6}$$

where x_i, y_i are M_{RBC} and $\frac{\text{iA1c}}{1000-\frac{2}{3}\text{iA1c}}$ of each participant, respectively.

The direct method – the k_g of each participant was calculated by the following equation:

$$k_g = \frac{\text{iA1c}}{(1000 - \frac{2}{3}\text{iA1c})M_{RBC}\text{AG}} \tag{7}$$

Then, average and standard deviation of each k_g was calculated.

2.5 Confirmation of derived k_g

The method of obtaining M_{RBC} from *AG* and iA1c was applied to data from three patients with latent hemolysis who were presented in a previous case studies [10–12].

Herranz [10] and Ishii's [11] data showed changes in HbA1c during the course of the study. Therefore, M_{RBC} was calculated separately for each period. For the Ishii case [11], AG was calculated by averaging self-monitoring of blood glucose (SMBG) data for each periodperiod. The Hiratani study [12] examined ^{51}Cr erythrocyte lifespan measurement during hospitalization in Oct 1999 and CGM in Feb 2016. While HbA1c and plasma glucose concentrations fluctuate routinely, RBC lifespan remain comparatively constant, especially when influenced by a certain diseases (stomatocytosis). Furthermore, supply of ^{51}Cr was ceased in Japan in 2015 and thus it can no longer by used to study erythrocyte lifespan.

3 Results

3.1 Participant characteristics

Participant demographics are shown in Table 1. All participants had no more than 16% GA. There was no significant difference in the GA of anemic and non-anemic subjects. However HbA1c, Hb, EC and their derivatives showed significant variation between the two groups.

The demographic information on the 3 patients from the previous cases are shown in Table 2.

3.2 Estimation of k_g

EC derived M_{RBC} and $\frac{\text{iA1c}}{1000-\frac{2}{3}\text{iA1c}}$ are shown in Figure 1. A linear relationship was successfully observed.

k_g calculated by the two methods outlined previously, for non-hemolytic participants and the entire study population are seen in Table 3. All 4 figures can be approximated to 7×10^{-6}. Figure 1 shows that data from severe hemolytic patients is less stable. Thus, the value derived from the direct method for calculating k_g is likely to be the least accurate. Excluding this value as an outlier, the 3 remaining figures were 6.94 – 6.99 $\times 10^{-6}$ (average 6.970×10^{-6}). Therefore, considering significant figures, k_g can be said to be 7.0×10^{-6}.

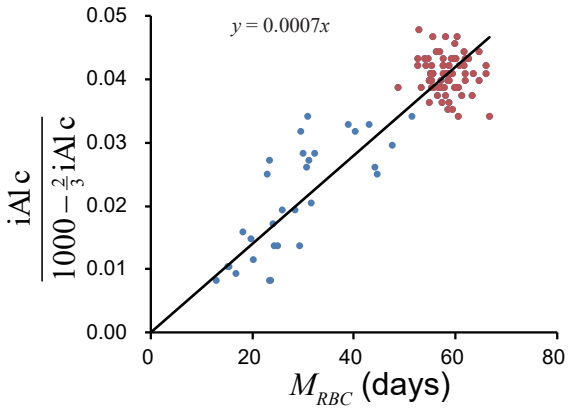

Figure 1. Relationship between EC derived M_{RBC} and iA1c/(1000−(2/3)iA1c). *Red circles* denote non-hemolytic participants and *blue circles* denote hemolytic patients. *Black line* denotes regression line through origin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1: Participants Characteristics

	non-hemolysis	hemolysis	p
n (M/F)	76 (30/46)	31 (17/14)	0.1463
Age (years)	62.3±7.9	45.6±15.0	1.37×10^{-6}
HbA1c (%)	5.78 ± 0.25	4.05 ± 0.78	2.27×10^{-13}
iA1c (mmol/mol)	39.7 ± 2.7	20.8 ± 8.5	2.27×10^{-13}
GA (%)	13.57 ± 1.07	13.06 ± 1.75	0.151
GA/iA1c	0.343 ± 0.032	0.762 ± 0.363	5.85×10^{-7}
Hb (g/dL)	14.26 ± 1.16	9.75 ± 1.97	2.44×10^{-14}
EC (μmol/g Hb)	1.40 ± 0.21	5.47 ± 2.13	1.42×10^{-11}
EC-M_{RBC} (days)	58.5 ± 3.41	29.0 ± 10.0	4.46×10^{-17}

Results are expressed as mean ± standard deviation (SD). Sex ratio was examined by χ^2 test. Other items were examined by t-test (bilateral). GA, glycated albumin; EC, erythrocyte creatine

Table 2: Characteristics of three reported patients with latent hemolysis and DM

Case	Herranz [10]	Ishii [11]	Hiratani [12]
Age/Sex	30F	72M	58F
Disease	AIHA	AIHA	HSt
DM	Type 1	Type 2	Type 2
HbA1c (%)	5.4	6.5	5.8
GA (%)	–	26.1	23.3
Hb (g/dL)	normal	13.5	11.5
Ret (%)	normal	1.3	1.3
Hpt (mg/dL)	normal	82	58

These patients showed normal Hb, reticulocyte, and haptogloblin. AIHA, autoimmune hemolytic anemia; HSt, hereditary stomatocytosis, DM, diabetes mellitus; GA, glycated albumin; Hb, hemoglobin; Ret, reticulocyte; Hpt, haptoglobin.

Table 3: k_g estimation

population	slope	straight forward
the whole	6.973×10^{-6}	$(7.073 \pm 1.229) \times 10^{-6}$
non-hemolytic	6.942×10^{-6}	$(6.994 \pm 0.662) \times 10^{-6}$

Results obtained by the direct method are expressed as mean ± standard deviation (SD).

3.3 Confirmation of derived k_g

The M_{RBC} using the derived k_g, 7.0×10^{-6} and M_{RBC} using ^{51}Cr half-life are shown in Table 4.

M_{RBC} derived from iA1c were 36.95±5.93, M_{RBC} derived from ^{51}Cr half-life were 41.29±2.22. Paired t-test: t, −0.9278; df, 2; p (bilateral), 0.4514. Thus, M_{RBC} derived from iA1c and M_{RBC} using ^{51}Cr half-life were not significantly different.

4 Discussion

Based on EC-derived M_{RBC} and HbA1c data, a more accurate value for the constant k_g was obtained. Though k_g was previously determined to be 6–10$\times 10^{-6}$ dL/mg/day [4], the more accurate value of 7.0×10^{-6} improves the usefulness of the proposed model allowing closer approximation of M_{RBC} based on AG and iA1c.

Moreover, the validity of k_g has been confirmed through comparison of M_{RBC} derived from iA1c and k_g with M_{RBC} derived from ^{51}Cr half-life. Of the three patients with hemolytic anemia and comorbid DM analyzed, data from two patients showed a remarkable correlation with the model derived figures. Data from one patient showed a 1.47 times difference in values however, this may be attributable to the use of SMBG instead of CGM, and the difficulty of standardizing ^{51}Cr data containing elution.

Variant hemoglobin should be distinguished from hemolysis when M_{RBC} determined by equation (1) is low. Glycated variant hemoglobin will exhibit different peaks in HPLC from normal HbA1c, resulting in erroneously low values for HbA1c (some variants show an artefactually high value). It has previously been reported that variant hemoglobin can be detected by the dissociation between HbA1c measured by HPLC and by immunoassay [13]. Moreover, some variant hemoglobins such as Hb Himeji [14] have different k_g values from normal Hb. In patients with these variant hemoglobins, equation (1) is likely to provide a falsely low M_{RBC}.

There are a number of limitations to this study. The data used to calculate a more specific estimate of k_g contained EC and HbA1c, but lacked CGM data, necessitating the use of . 100 mg/dL as an approximation of AG. However, participants were confirmed to be free of DM through GA, an indicator of glycemic control that is independent of mean erythrocyte age, with a cut off of GA no more than 16%. Further study with more complete data including CGM, HbA1c and EC would provide an even more definitive value for k_g. Another limitation is that the value for k_g derived in this study is totally dependent on equation (2) that derives M_{RBC} from EC. This equation was based on old published data [15], which used less sensitive and poorly specific chemical methods of measuring creatine which were prone to cross-reactivity with other guanidino compounds. This may reduce the reliability of the system. In contrast, in this study creatine was measured using an enzymatic method which was sensitive and specific to creatine in erythrocytes which uses 10-*N*-methylcarbamoyl-3,7-*bis* (dimethylamino) phenothiazine (MCDP), an *N*-methylcarbamoyl derivative of methylene blue, with a high molar absorption coefficient (9.6×10^7L mol^{-1} cm^{-1}) [16], as a chromogen.

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Declaration of interest

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References

[1] Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *N Engl J Med* . 1976;295:417–420. doi:10.1056/NEJM197608192950804.

[2] American Diabetes Association. Glycemic targets in *Standards of Medical Care in Diabetes – 2017. Diabetes Care* . 2017;40:S48–S56. doi:10.2337/dc17-S009.

[3] Panzer S, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak R. Glycosylated hemoglobins (GHb): an index of red cell survival. *Blood* . 1982;59:1348–1350.

Table 4: M_{RBC} of 3 cases in literature

Case	Herranz [10]		Ishii [11]		Hiratani [12]
HbA1c (%)	5.8	4.9	6.0	6.7	6.5
iA1c (mmol/mol)	39.9	30.0	42.3	50.2	47.5
AG (mg/dL)	203	148	138	177	184
iA1c derived M_{RBC}	28.7	29.6	45.3	41.9	38.1
(days)	29.2		43.6		38.1
^{51}Cr half-life (days)	20		20		17.8
^{51}Cr derived M_{RBC} (days)	42.9		42.9		38.1

HbA1c value is different from Table 2. The AG in Herranz [10] and Ishii [11] were calculated from blood glucose values using self-monitoring of blood glucose (SMBG).

[4] Kameyama M, Takeuchi S, Ishii S. Steady-state relationship between average glucose, HbA1c and RBC lifespan. *J Theor Biol* . 2018;447:111–117. doi:10.1016/j.jtbi.2018.03.023.

[5] Kameyama M, Koga M, Okumiya T. A novel method for calculating mean erythrocyte age using erythrocyte creatine. *bioRxiv* . 2019; 642942. doi:10.1101/642942.

[6] Hoelzel W, Weykamp C, Jeppsson JO, et al. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* . 2004;50:166–174. doi:10.1373/clinchem.2003.024802.

[7] Tsujino D, Nishimura R, Taki K, Miyashita Y, Morimoto A, Tajima N. Daily glucose profiles in Japanese people with normal glucose tolerance as assessed by continuous glucose monitoring. *Diabetes Technol Ther* . 2009; 11:457–460. doi:10.1089/dia.2008.0083.

[8] Malka R, Nathan DM, Higgins JM. Mechanistic modeling of hemoglobin glycation and red blood cell kinetics enables personalized diabetes monitoring. *Sci Transl Med* . 2016;8:359ra130. doi:10.1126/scitranslmed.aaf9304.

[9] Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. *Diabetes Care* . 2008;31:1473–1478. doi:10.2337/dc08-0545.

[10] Herranz L, Grande C, Janez M, Pallardo F. Red blood cell autoantibodies with a shortened erythrocyte life span as a cause of lack of relation between glycosylated hemoglobin and mean blood glucose levels in a woman with type 1 diabetes. *Diabetes Care* . 1999;22:2085–2086. doi:10.2337/diacare.22.12.2085.

[11] Ishii C, Tane N, Negishi K, Katayama S. A case of type 2 diabetes who showed discrepancy between plasma glucose and HbA1c due to latent autoimmune hemolytic anemia (in Japanese). *J Japan Diab Soc* . 2001; 44:157–160. doi:10.11213/tonyobyo1958.44.157.

[12] Hiratani K, Natazuka T, Suemori S, Wada H, Koga M. A case of stomatocytosis in a type 2 diabetic patient accompanied with falsely low HbA1c levels due to latent hemolysis (in Japanese). *J Japan Diab Soc* . 2016;59:719–723. doi:10.11213/tonyobyo.59.719.

[13] Miyazaki A, Kohzuma T, Kasayama S, Koga M. Classification of variant forms of haemoglobin according to the ratio of glycated haemoglobin to glycated albumin. *Ann Clin Biochem* . 2012;49:441–444. doi:10.1258/acb.2012.011192.

[14] Koga M, Inada S, Shimizu S, Hatazaki M, Umayahara Y, Nishihara E. Aldimine formation reaction, the first step of the maillard early-phase reaction, might be enhanced in variant hemoglobin, Hb Himeji. *Ann Clin Lab Sci* . 2015;45:643–649.

[15] Fehr J, Knob M. Comparison of red cell creatine level and reticulocyte count in appraising the severity of hemolytic processes. *Blood* . 1979;53:966–976.

[16] Okumiya T, Jiao Y, Saibara T, et al. Sensitive enzymatic assay for erythrocyte creatine

with production of methylene blue. *Clin Chem* . 1998;44:1489–1496.