Age-Adjusted Glycated Albumin at Diagnosis is more Correlated with the Product of Age and Plasma Glucose than Plasma Glucose Alone in Patients with Neonatal Diabetes Mellitus

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Background: We previously showed that glycated albumin (GA) is a useful glycemic control indicator in patients with neonatal diabetes mellitus (NDM), and that age-adjusted GA (Aa-GA) can reflect more accurately glycemic control status. Here, we investigated whether the age at diagnosis influences Aa-GA at diagnosis of NDM. **Methods:** Eight patients with NDM whose GA was measured at diagnosis (age at diagnosis: 39 ± 18 days; GA: $31.3 \pm 7.6\%$; Aa-GA: $47.1 \pm 10.3\%$; plasma glucose: 525 ± 194 mg/dl) were included. Aa-GA was calculated as follows: $\text{Aa-GA} = \text{GA} \times 14.0/[1.77 \times \log\text{-age (days)} + 6.65]$. Correlations of GA or Aa-GA at diagnosis with its logarithmically transformed age in days (log-age), plasma glucose, and their product

were investigated. **Results:** GA at diagnosis was not significantly correlated with log-age or plasma glucose. On the other hand, Aa-GA at diagnosis was significantly positively correlated with plasma glucose ($R = 0.75$, $P = 0.031$) and was more strongly positively correlated with the product of plasma glucose and log-age ($R = 0.82$, $P = 0.012$) although it was not correlated with log-age. **Conclusion:** Aa-GA at diagnosis is influenced by both age in days and plasma glucose. This finding is likely to show the aspect that age in days is almost equal to diabetes duration because glycemic control indicators including GA reflect the weighted mean of plasma glucose. *J. Clin. Lab. Anal.* **30**:1086–1091, 2016. © 2016 Wiley Periodicals, Inc.

Key words: diabetes duration; glycated albumin; neonatal diabetes mellitus; plasma glucose

INTRODUCTION

Neonatal diabetes mellitus (NDM) is a type of diabetes mellitus which is caused by single-gene abnormality, and develops in the insulin-dependent status within 6 months after birth (1). NDM is classified into

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two major categories: (a) transient NDM (TNDM) in which treatment will become unnecessary within several months after onset, and (b) permanent NDM (PNDM) which requires lifelong treatment. The main cause of TNDM is overexpression of the imprinted region of chromosome 6q24 which is expressed from the paternal chromosome; most cases of TNDM develop within 1 week after birth and resolve within 3 months on average (2, 3). The main cause of PNDM is abnormality of the *KCNJ11* gene which constitutes the ATP-sensitive potassium channel of pancreatic β cells; PNDM develops at 7 weeks after birth on average (3, 4).

It is known that patients with diabetes mellitus are associated with increased glycation of various proteins compared with those without diabetes mellitus, and it is suggested that some of these glycated proteins contribute to the onset and progression of diabetic complications (5). Of such glycated proteins, HbA1c is frequently used in clinical settings as a glycemic control indicator (6, 7). It is known that patients with a high level of hemoglobin F (HbF) show an apparently low level of HbA1c (8, 9). HbF is the main hemoglobin during the fetal period; HbF accounts for 80–90% of hemoglobin immediately after birth, whereas hemoglobin A (HbA) accounts for only 10–20% of hemoglobin (10). After birth, HbF is gradually replaced by HbA; by 6 months after birth, HbA accounts for the largest proportion of hemoglobin. As neonates show an apparently low level of HbA1c because of the influence of HbF, HbA1c cannot be used as a glycemic control indicator for patients with NDM (11).

Similar to HbA1c, glycated albumin (GA) is used as a glycemic control indicator (12). Because GA reflects plasma glucose during a shorter period than HbA1c, it can be used to promptly evaluate the therapeutic effect at the start of treatment of diabetes mellitus. We previously reported that GA is a useful glycemic control indicator for patients with NDM because GA is not influenced by a high level of HbF (11). However, it was found that neonates show an apparently low level of GA because albumin metabolism is increased in neonates (13, 14). On the other hand, GA is positively correlated with logarithmically transformed age in days (log-age); therefore, in order to evaluate glycemic control in patients with NDM by using GA, it is necessary to compare GA with the reference value for the age of the patient (14). In addition, we reported that age-adjusted GA (Aa-GA) is applicable to all ages with the reference value for adults and that it is possible to evaluate glycemic control by using Aa-GA (15). Indeed, we showed that Aa-GA accurately reflects blood glucose in patients with NDM by comparing it

with calculated glycated albumin determined by past blood glucose concentrations (16).

Glycemic control indicators (HbA1c or GA) reflect plasma glucose according to the life span of the indicator (17). Therefore, if plasma glucose level is not stable for a period of at least two to three times the half-life, the level of the glycemic control indicator fluctuates as well. On the other hand, because glycemic control indicators reflect the weighted mean of the plasma glucose level during the period (17, 18), they are also influenced by a temporal factor (i.e., for how long of the life span of the glycated protein did plasma glucose level fluctuate?).

NDM is a congenital diabetes mellitus due to gene abnormality; in patients with NDM, plasma glucose starts to increase after birth; therefore, age at the time of diagnosis is estimated as diabetes duration, and glycemic control indicators may be influenced not only by plasma glucose level at diagnosis but also by age. Therefore, in this study, we investigated whether the age at diagnosis in patients with NDM affects Aa-GA at diagnosis in them.

MATERIALS AND METHODS

Eight patients with NDM whose GA was measured at diagnosis were included in this study (Table 1). Six patients (Case 1–Case 6) were from previous reports (11, 19–21), and the other two patients (Case 7 and Case 8) were newly diagnosed patients. For the newly diagnosed patients, genetic analysis was performed for known responsible genes. The Ethics Committee of Asahikawa Medical University approved the gene analysis and written informed consent was obtained from the parents of the patients.

Plasma glucose was determined using the hexokinase, glucose-6-phosphate dehydrogenase method. GA was determined by a Hitachi 7600 autoanalyzer (Hitachi Instruments Service Co., Tokyo, Japan), by the enzymatic method using albumin-specific proteinase, ketoamine oxidase, and albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma Co., Tokyo, Japan) (22). The reference range of GA in adults is between 11.7% and 16.2% (23). Because glycated proteins as glycemic control indicators (e.g., HbA1c, GA) are influenced by the weighting function of past plasma glucose (17, 18), GA is positively correlated with logarithmically transformed duration of hyperglycemia. Therefore, log-age was used in this study. Aa-GA was calculated by the following formula described in a previous report (15).

$$\text{Aa-GA (\%)} = \text{GA (\%)} \times 14.0 / [1.77 \times \log\text{-age (days)} + 6.65]$$

The results were expressed as mean $\pm$ SD. To assess the normality of data, the Shapiro–Wilk test was

TABLE 1. Clinical characteristics of study patients

No.	Sex	Type	Etiology	At birth		At diagnosis			
				Gestation (weeks)	BW (g)	Age (days)	PG (mg/dl)	GA (%)	Aa-GA (%)
1	M	PNDM	<i>KCNJ11</i>	41	2582	53	855	40.8	59.5
2	F	PNDM	PH	37	1353	58	349	31.7	45.9
3	M	PNDM	<i>KCNJ11</i>	39	2640	45	346	27.3	40.3
4	F	PNDM	<i>KCNJ11</i>	38	1856	17	468	24.8	39.8
5	M	PNDM	<i>KCNJ11</i>	39	2308	49	703	45.1	66.2
6	M	TNDM	pUPD6	38	2352	21	340	26.9	42.4
7	F	TNDM	Unknown	38	2530	50	472	25.3	37.1
8	F	PNDM	<i>KCNJ11</i>	39	1998	15	664	28.1	45.6
				39 ± 1	2200 ± 441	39 ± 18	525 ± 194	31.3 ± 7.6	47.1 ± 10.3

BW, birth weight; PG, plasma glucose; GA, glycated albumin; Aa-GA, age-adjusted GA; PNDM, permanent neonatal diabetes mellitus; PH, pancreas hypoplasia; TNDM, transient neonatal diabetes mellitus; pUPD6, paternal uniparental disomy of chromosome 6. All numerical values are given as mean ± SD.

performed for each variable. To assess the correlation among variables, the Pearson's correlation coefficient (R) was calculated if variables are normally distributed and the Spearman rank-order correlation coefficient (ρ) was calculated if variables are not normally distributed. The significance of differences between groups was calculated using a Student's t -test if a parameter is normally distributed. All analyses were performed using SPSS 20 (Tokyo, Japan), and $P < 0.05$ was considered significant.

RESULTS

The clinical characteristics of the eight patients with NDM were as follows: sex: four males and four females; disease type: two patients with TNDM and six patients with PNDM (Table 1). Age at diagnosis was 39 ± 18 days and plasma glucose at diagnosis was 525 ± 194 mg/dl. GA and Aa-GA at diagnosis were as high as $31.3 \pm 7.6\%$ and $47.1 \pm 10.3\%$, respectively. In more than half of study patients, the cause of NDM was abnormality of the *KCNJ11* gene.

No significant difference in Aa-GA at diagnosis between sex was observed (males: $52.1 \pm 12.7\%$; females: $42.1 \pm 4.4\%$; $P = 0.188$); Aa-GA at diagnosis was not significantly correlated with gestational week ($\rho = 0.426$, $P = 0.293$) or birth weight ($R = 0.114$, $P = 0.788$). GA at diagnosis was not significantly correlated with log-age or plasma glucose (log-age: $\rho = 0.452$, $P = 0.260$; plasma glucose: $\rho = 0.548$, $P = 0.160$). Aa-GA at diagnosis was not significantly correlated with log-age ($\rho = 0.333$, $P = 0.420$) but was significantly correlated with plasma glucose ($R = 0.752$, $P = 0.031$) (Fig. 1). Aa-GA was significantly correlated with the product of log-age and plasma glucose ($R = 0.824$, $P = 0.012$), and the correlation coefficient for the

Fig. 1. Correlations of age-adjusted GA (Aa-GA) at diagnosis with log-age (a) and plasma glucose (b) in patients with NDM.

product was larger than that for plasma glucose alone (Fig. 2). No significant correlation was observed between plasma glucose and log-age ($\rho = 0.500$, $P = 0.207$).

DISCUSSION

Recently, we found that Aa-GA is a more accurate blood glucose indicator in patients with NDM based on the fact that mean blood glucose has strong positive correlation with Aa-GA (16). In this study, we obtained the new findings that Aa-GA at diagnosis in the patients with NDM is potentially affected by the age of diagnosis. That is, Aa-GA at diagnosis in the patients with NDM was significantly correlated with the product of log-age and plasma glucose at that time, and the correlation coefficient for the product was larger than that for plasma glucose alone although Aa-GA at diagnosis was not significantly correlated

Fig. 2. Correlation of age-adjusted GA (Aa-GA) at diagnosis with the product of log-age and plasma glucose in patients with NDM.

with log-age but was significantly correlated with plasma glucose.

It is considered that patients with NDM show impaired insulin secretion from the fetal period because NDM is a disease associated with impaired insulin secretion due to gene abnormality; therefore, NDM is frequently accompanied by intrauterine growth retardation (1). In this study as well, the mean birth weight was as low as 2202 ± 440 g. Although insulin does not cross the placenta, glucose is transferred between mother and fetus through glucose transporter (GLUT) type 1 or type 3 which are located in the placenta (24, 25). Therefore, plasma glucose of patients with NDM during the fetal period remains at similar level to that of the mother, and hyperglycemia does not develop. After delivery, the regulatory mechanism of plasma glucose through the placenta disappears, and therefore plasma glucose increases. That is to say, because patients with NDM manifest hyperglycemia due to impaired insulin secretion from the time of birth, age in days is considered to roughly reflect diabetes duration. For these reasons, it is considered that Aa-GA of patients with NDM showed a stronger significant correlation with the product of plasma glucose and log-age than with plasma glucose alone. In considering possible explanations for this result, it seems likely that more recent plasma glucose levels have a stronger effect on GA levels than older ones, whose influence on GA levels decline exponentially with time because glycated proteins including GA or Aa-GA reflect weighted average of plasma glucose.

Because age in days reflects diabetes duration in patients with NDM, it is possible to determine diabetes duration with a certain degree of accuracy. However, diabetes duration may actually be shorter than age in days. For example, neonates may show hypoglycemia reflecting immature gluconeogenesis after birth. Accordingly, there are reports on patients with NDM who had hypoglycemia or normoglycemia for a certain period after birth. Nishizawa et al. reported on

monozygotic twin sisters with NDM; their plasma glucose at birth was as low as 40 and 46 mg/dl, respectively (26). Plasma glucose at birth of Case 4 in this study was 88 mg/dl, which was within the reference range (19). It is estimated that in such patients, hyperglycemia develops after a certain period after birth. Therefore, the variation in the duration of hypoglycemia among patients may be one of the causes of the absence of a significant correlation between age in days and Aa-GA. On the other hand, it is suspected that some patients with NDM show a relatively low level of Aa-GA compared with plasma glucose level. It is known that the birth weight of patients with NDM is often low (1, 3). Because low birth weight babies have increased albumin metabolism compared with normal weight babies (27), Aa-GA in low birth weight babies with NDM may be lower than that in normal weight babies. It is considered that if these tendencies can be adjusted, there might be a significant correlation between Aa-GA at diagnosis and log-age, and there might also be a stronger significant correlation between Aa-GA at diagnosis and the product of plasma glucose and log-age.

Glycemic control indicators are influenced by diabetes duration. For example, patients with fulminant type 1 diabetes mellitus (FT1DM) in which plasma glucose rapidly increases, show marked hyperglycemia and ketoacidosis at diagnosis; however, because diabetes duration is short, HbA1c, a medium- to long-term glycemic control indicator, is normal or only slightly high (28, 29). Relatively low HbA1c level in spite of marked hyperglycemia is one of the diagnostic criteria for FT1DM (29, 30). In autoimmune type 1 diabetes mellitus (T1ADM), on the other hand, plasma glucose at diagnosis is lower than that in FT1DM, whereas diabetes duration is longer than that in FT1DM; thus, high HbA1c level is observed in T1ADM (28). It is therefore conceivable that the difference in HbA1c level between FT1DM and T1ADM is due to the difference in diabetes duration.

However, it is not easy to estimate diabetes duration; in general, it can be roughly considered as the duration from the onset of diabetic symptoms (29). We reported that in patients with FT1DM, GA at the time of onset is significantly positively correlated with urinary C peptide immunoreactivity (CPR) (31). In other words, it is considered that in FT1DM, the lower the urinary CPR which reflects function of insulin secretion, the shorter the latency to the onset of diabetic ketoacidosis, resulting in a low GA at the time of onset. However, neither GA nor urinary CPR was significantly correlated with actual diabetes duration (31), suggesting that diabetes duration is not accurately determined in patients with FT1DM.

It is more difficult to determine accurately diabetes duration of patients with type 2 diabetes. If the duration of hyperglycemia is extended (generally, by at least two to three times the half-life of a glycemic control indicator), the glycemic control indicator will remain in a certain range. Accordingly, it is considered that because type 2 diabetes mellitus is associated with a gradual increase in plasma glucose resulting in long disease duration before diagnosis, glycemic control indicators before treatment are not influenced by diabetes duration. In addition, most patients with type 2 diabetes mellitus are asymptomatic.

The phenomenon that glycemic control indicators are significantly correlated with the product of log-age (diabetes duration) and plasma glucose in NDM has not been reported for other types of diabetes mellitus; as far as we know, this is the first report on this finding. However, only a small number of patients with NDM (eight patients) were investigated in this study. In the future, it is necessary to review the current finding using a large number of patients with NDM. NDM is a unique type of diabetes mellitus whose diabetes duration can be determined by age in days, an objective indicator, not by symptoms. Further study utilizing this characteristic is necessary.

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