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CASE REPORT

Cases of fulminant type 1 and type 2 diabetes mellitus whose HbA1c levels were unmeasurable due to increased labile HbA1c

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Abstract

Although the measurement of hemoglobin A1c (HbA1c) using high-performance liquid chromatography (HPLC) is routinely used to estimate average blood glucose levels, it may not be accurately measured for various reasons, such as alteration of red blood cell lifespan and the existence of hemoglobin variants; including hemoglobin F (HbF). Here, we report cases of fulminant type 1 and type 2 diabetes mellitus in which HbA1c levels were unmeasurable because of increased labile HbA1c levels. Case 1 involved a 73-year-old man with fulminant type 1 diabetes mellitus, who was brought to our hospital with diabetic ketoacidosis. The patient's blood glucose level was 994 mg/dL, and HbA1c was unmeasurable, which turned out to be 6.2% on the next day when the blood glucose level was normalized. Case 2 involved a 72-year-old man with type 2 diabetes mellitus, whose blood glucose level was 767 mg/dL, and HbA1c was unmeasurable, which turned out to be 17.9% the following day. In both cases, the chromatograms showed that the HbA1c peaks overlapped with large labile HbA1c peaks, which decreased the next day. It is important to keep in mind that HbA1c levels may not be accurately measured in cases of extreme hyperglycemia because of an increase in labile HbA1c, regardless of the absolute HbA1c level.

Keywords Labile HbA1c · HPLC · Hyperglycemia · Fulminant type 1 diabetes · Type 2 diabetes

Introduction

Hemoglobin A1c (HbA1c) is used to estimate past blood glucose levels based on the percentage of hemoglobin in red blood cells that undergo glycation during their lifespan of approximately 120 days [1]. It is a useful parameter that reflects the average blood glucose level over the past 1–2 months in the treatment of diabetes mellitus [2]. However, various factors other than blood glucose can affect the measurement of HbA1c levels, and it is important to bear in mind that HbA1c levels do not always correctly reflect blood glucose levels [3]. High-performance liquid chromatography (HPLC) is the most commonly used method for HbA1c [4, 5]. Using chromatograms, we can examine the fractions of HbA1c and speculate why HbA1c levels do not correctly reflect blood glucose levels. Recently, we encountered cases of fulminant type 1 and type 2 diabetes mellitus in which HbA1c levels were unmeasurable using HPLC due to increased labile HbA1c levels. Labile HbA1c is formed by an extremely high concentration of glucose and may prevent the accurate measurement of HbA1c. Therefore, caution should be exercised when evaluating HbA1c levels in cases of extreme hyperglycemia, particularly in fulminant type 1 diabetes, characterized by an abrupt onset of hyperglycemia with relatively low HbA1c levels.

Case description

The first case was that of a 73-year-old man who had not undergone a health checkup for more than 10 years. Two days before admission, he started to feel fatigued at around noon, had frequent urination and thirst in the evening, and started vomiting at night. Upon admission, the patient was diagnosed with diabetic ketoacidosis with a blood glucose level of 1,272 mg/dL, pH of 7.0, HCO_3^- 4.9 mmol/L, and positive urinary ketone bodies, and was admitted to our hospital. He had no special medical or family history and was

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not taking any medication. He had no history of obesity or alcohol consumption and had been a non-smoker since the age of 30 years (10 pack-years). He had regular meals three times a day and did not consume any sugary drinks. On physical examination, he was 158 cm, 44.6 kg with a body mass index (BMI) of 17.0 kg/m^2, body temperature 36.9 °C, blood pressure 86/59 mmHg, pulse 98/min, and Japan Coma Scale I-1. Blood tests showed a glucose level of 994 mg/dL and HbA1c was unmeasurable (Table 1). Arterial blood gas analysis indicated metabolic acidosis. Urinary ketone bodies were positive, and blood ketone levels were higher than normal. In addition, the white blood cell, C-reactive protein, blood urea nitrogen, creatinine, and uric acid levels were higher, and sodium and hemoglobin levels were lower than normal. High amylase (2,615 IU/L) and lipase (99 IU/L) levels were also noted, indicative of fulminant type 1 diabetes.

We initiated a massive infusion of fluids and continuous insulin infusion, and the dehydration and hyperglycemia gradually improved. The next day, the insulin treatment was switched from continuous infusion to subcutaneous injection. HbA1c level was retested after his blood glucose levels were almost normalized, which turned out to be 6.2%. His glycated albumin level was 20.1%, which was consistent with a relatively low HbA1c level despite an extremely high glucose level. The HPLC chromatogram of HbA1c upon admission indicated a large peak of labile HbA1c eluted earlier than stable HbA1c (Fig. 1). The peak dramatically decreased the next day, consistent with the rapid turnover of labile HbA1c [6, 7] (Fig. 1). The fasting serum C-peptide level was below the sensitivity limit. The serum C-peptide levels after glucagon challenge were lower than the sensitivity limits. Both glutamic acid decarboxylase (GAD) antibody and islet antigen-2 (IA-2) antibodies were negative. The patient was diagnosed with fulminant type 1 diabetes mellitus according to the criteria of the Japan Diabetes Society [8]. Regarding diabetic complications, neuropathy and retinopathy were not observed; however, microalbuminuria was observed (urinary albumin, 106 mg/g creatinine). He was discharged 18 days later with a self-injection of 17 units/day of insulin aspart and 9 units/day of insulin degludec.

The second case was that of a 72-year-old man. He regularly visited a local general hospital for the treatment of atrial fibrillation, chronic heart failure, hypertension, dyslipidemia, hyperuricemia, and osteoarthritis but had never been tested for blood glucose levels. In a screening conducted before the treatment of the colon polyps, the patient was diagnosed with diabetes mellitus. One day before admission, his blood glucose level was 714 mg/dL, and intravenous

Table 1 Laboratory data of the two cases

	Case 1	Case 2	Reference		Case 1	Case 2	Reference
Hematology				Biochemistry			
WBC ($\times 10^3$/μl)	34.6	5.6	3.3–8.6	BUN (mg/dL)	84.1	21.6	8.0–20.0
Hb (g/dL)	12.8	12.1	13.7–16.8	Cre (mg/dL)	2.68	1.02	0.65–1.07
Plt ($\times 10^4$/μl)	24.7	16.6	15.8–34.8	eGFR (mL/min/1.73 m^2)	19.3	55	
Diabetes				UA (mg/dL)	11.6	4.9	3.6–7.0
Glucose (mg/dL)	994	767	70–109	AST (IU/L)	28	24	13–30
HbA1c (%)	UM (6.2)	UM (17.9)	4.6–6.2	ALT (IU/L)	18	36	10–42
GA (%)	20	65.3	11–16	γGTP (IU/L)	11	26	13–64
S-CPR (ng/ml)	ND	2.08	0.69–2.45	T-Bil (mg/dL)	0.9	1.1	0.4–1.5
U-CPR (μg/day)	ND	12.8		TP (g/dL)	6.6	6.9	6.6–8.1
GAD-antibody	–	–		Alb (g/dL)	3.9	4.2	4.1–5.1
Urinalysis				Na (mEq/L)	126	127	138–145
Glucose	4+	4+		K (mEq/L)	4.8	4.6	3.6–4.8
Protein	–	–		Cl (mEq/L)	86	90	101–108
Ketone	2+	–		TG (mg/dL)	74	185	30–150
Arterial blood gas analysis				HDL-C (mg/dL)	73	77	40–63
pH	7.20	NT	7.35–7.45	LDL-C (mg/dL)	74	118	
pO_2 (mmHg)	110		80–100	Amylase (IU/L)	2,615	88	44–132
pCO_2 (mmHg)	17		35–45	Lipase (IU/L)	99	NT	13–55
HCO_3^- (mEq/L)	15.1		22–26	CRP (mg/dL)	5.25	NT	0.00–0.14

WBC white blood cells, *Hb* hemoglobin, *Plt* platelets, *HbA1c* hemoglobin A1c, *GA* glycoalbumin, *S-CPR* serum C-peptide, *U-CPR* urine C-peptide, *GAD-antibody* glutamic acid decarboxylase antibody, *BUN* blood urea nitrogen, *Cre* creatinine, *eGFR* estimated glomerular filtration rate, *UA* uric acid, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γGTP* gamma-glutamyl transferase, *T-Bil* total bilirubin, *TP* total protein, *Alb* albumin, *Na* sodium, *K* potassium, *Cl* chloride, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *CRP* C-reactive protein, *ND* not detected, *UM* unmeasurable, *NT* not tested

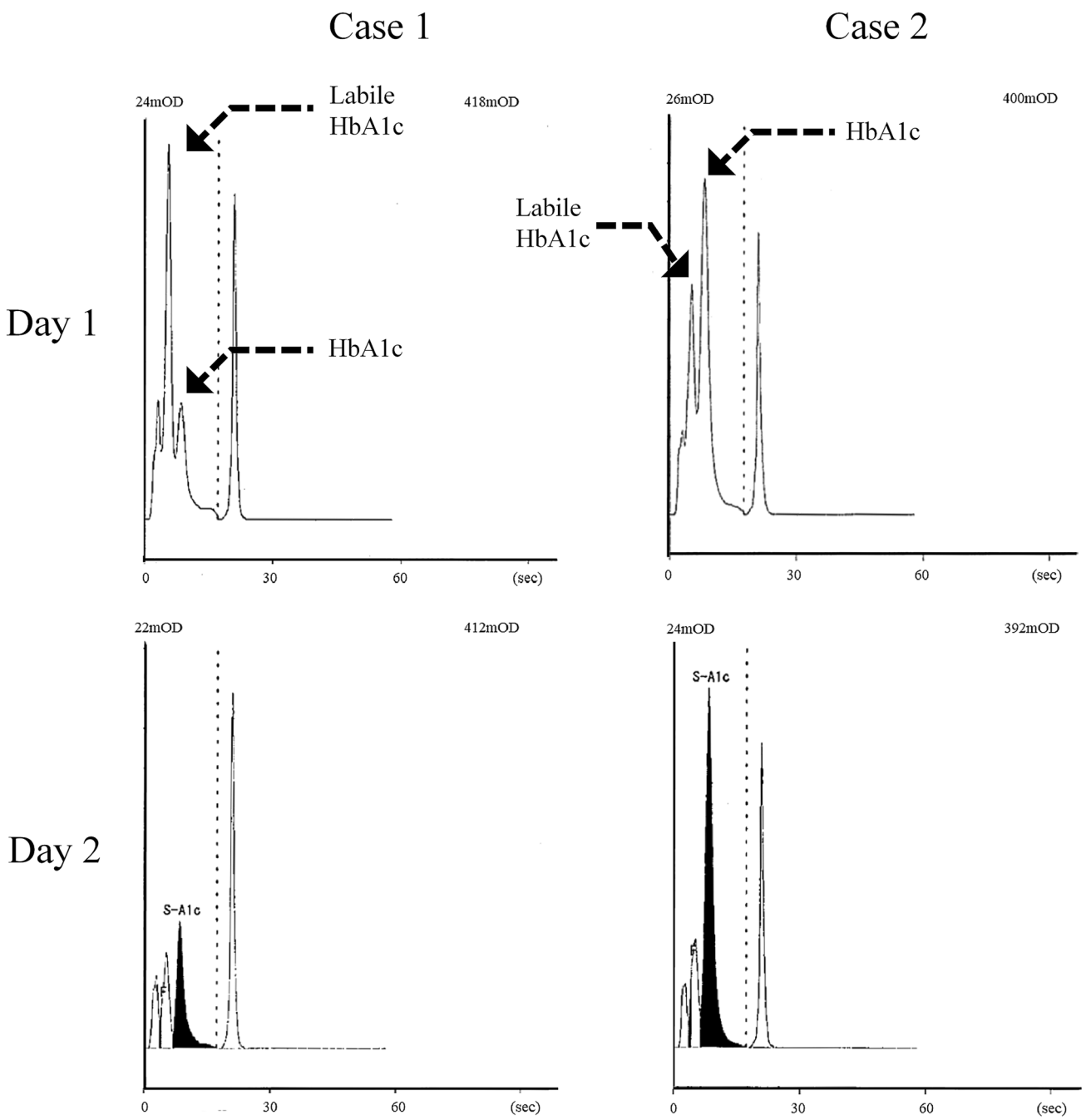

Fig. 1 Chromatograms of the HPLC analysis of HbA1c in case 1 and case 2 (left column, case 1; right column, case 2. Top row, day 1; bottom row, day 2)

insulin infusion and subcutaneous injection of 7 units of insulin glargine were administered. The following day, he was referred to our hospital. He had been experiencing thirst, polydipsia, and polyuria for a month. His weight had decreased by 10 kg over the previous 6 months. The patient had no relevant family histories. He was taking angiotensin II receptor blockers, beta-blockers, calcium channel blockers, loop diuretics, statins, eicosapentaenoic acid, direct oral anticoagulants, aspirin, proton pump inhibitors, and non-steroidal anti-inflammatory drugs. His maximum weight was 84 kg (BMI 28.7 kg/m^2) a year ago. He had no history of alcohol consumption. He had been a non-smoker since the age of 40 (20 pack-years). He had regular meals three times a day and drank approximately two cups of sugary drinks per day. On physical examination, he was 171 cm, 69.3 kg with a BMI of 23.7 kg/m^2, body temperature 35.9 °C, blood

pressure 90/62 mmHg, pulse 96/min, and Japan Coma Scale was 0. The next day, blood tests showed that blood urea nitrogen and triglyceride levels were higher, and sodium levels and estimated glomerular filtration rate (eGFR) were lower than normal (Table 1). His blood glucose level was 767 mg/dL, his HbA1c level was unmeasurable, and his glycated albumin level was 65.3%. Urinalysis results were negative for ketone bodies. Intensive insulin therapy was initiated with multiple injections. On day 2, HbA1c was retested after his blood glucose levels had almost normalized and were found to be 17.9%. The HPLC chromatograms of HbA1c upon admission showed a large peak of labile HbA1c that eluted earlier than stable HbA1c, which decreased the next day (Fig. 1). Fasting serum C-peptide and urine C-peptide levels were decreased, but detected, and both GAD and IA-2 antibodies were negative. Abdominal echography revealed no obvious pancreatic abnormalities. Therefore, the patient was diagnosed with type 2 diabetes mellitus. In addition, the patient had a weak Achilles tendon reflex and orthostatic hypotension. No obvious evidence of diabetic retinopathy or nephropathy was observed. He was discharged 13 days later with a self-injection of 26 units/day of insulin aspart and 2 units/day of insulin glargine.

Discussion

HbA1c levels are used in the management of patients with diabetes. The formation of HbA1c consists of two steps. First, the aldehyde group of glucose forms a reversible Schiff base linkage with the N-terminus of the β-chain of hemoglobin to form labile HbA1c (or pre A1c) [6, 9]. Second, labile HbA1c undergoes Amadori rearrangement to form stable ketoamine (HbA1c). The conversion of non-glycated hemoglobin (HbA) to labile HbA1c is a rapid and reversible reaction, whereas the conversion of labile HbA1c to stable HbA1c is a slow and irreversible reaction [6, 7]. The HbA1c levels of the two patients were judged as unmeasurable because a huge peak of labile HbA1c was formed in the HPLC chromatogram, which interfered with the accurate measurement of the stable HbA1c fraction. When HbA1c levels were retested the day after blood glucose levels were normalized, labile HbA1c peaks dramatically decreased in both cases. This phenomenon is consistent with the reported rapid interconversion between labile HbA1c and non-glycated hemoglobin (HbA) [9]. To the best of our knowledge, this is the first case report on labile HbA1c levels in patients with fulminant type 1 diabetes. We speculate that the measurement of HbA1c in patients with fulminant type 1 diabetes mellitus may be more susceptible to interference by labile HbA1c than other types of diabetes because patients with fulminant type 1 diabetes mellitus usually have extremely high glucose levels and relatively low HbA1c levels due to the sudden onset of the disease.

Labile HbA1c has been reported to be positively correlated with blood glucose levels in patients with both type 1 and type 2 diabetes [10]. Another study suggested that a highly labile HbA1c/HbA1c ratio is associated with increased blood glucose levels, anemia, chronic liver disease, and renal disease [11]. Recently, the usefulness of labile HbA1c as a marker reflecting a rapid increase in blood glucose levels has been reported [12]. Considering the acute onset of this disease, we expect labile HbA1c to be useful for the diagnosis of fulminant type 1 diabetes mellitus.

Two companies, Arkray, Inc. and Tosoh Bioscience Inc., provide HPLC devices for the measurement of HbA1c in Japan [13] (Table 2). The device used in our hospital was purchased from Arkray, Inc. (ADAMS A1c HA-8190V). This device calls the fraction corresponding to labile HbA1c as #C, and it judges HbA1c as "unmeasurable" when the #C fraction exceeds 4%. According to Arkray's basic data collection, peak #C exceeds 4% when blood glucose is approximately 500–1000 mg/dL. On the other hand, Tosoh's devices do not have a default setting of an error notification for high levels of the fraction corresponding to labile HbA1c (called "L-A1c" in their

Table 2 Features of major devices for the measurement of HbA1c in Japan

Method	Device	Manufacturer	Interference by labile HbA1c	Default setting of the fraction corresponding to labile HbA1c for an error report
HPLC	ADAMS A1c HA-8190V ADAMS A1c HA-8182	Arkray, Inc	Possible	Over 4% [1]
HPLC	HLC723G11 HLC723G9	Tosoh Bioscience Inc	Possible	None [2]
Enzymatic assay	MetaboLead HbA1c	Minaris Medical Co., Ltd	None [3]	Not applicable
Immunoassay	Determiner L HbA1c	Minaris Medical Co., Ltd	None [4]	Not applicable

References: [1] Internal data, ADAMS A1c HA-8190V, Arkray, Inc., as of November 2019. [2] User's manual, HLC723G11, Tosoh Bioscience Inc., November 2014 [3] User's manual, MetaboLead HbA1c, Minaris Medical Co., Ltd., July 2021 [4] Internal data, Determiner L HbA1c, Minaris Medical Co., Ltd., as of May 2022

devices). Although an increase in labile HbA1c reportedly does not affect the measurement of stable HbA1c unless the blood glucose level exceeds 1000 mg/dL in the Tosoh's devices, we may have to consider the possibility that reported HbA1c values could be interfered by the labile HbA1c peak, particularly in patients of fulminant type 1 diabetes with extremely high blood glucose and relatively low HbA1c levels.

In addition to HPLC, enzymatic and immunoassay methods have been used to measure HbA1c [14, 15]. The measurement of HbA1c using these methods was not affected by the labile HbA1c levels (Table 2). Glycated albumin is also useful in this regard [16]. In both cases in this report, glycated albumin levels were measurable, and the results were reasonable compared to the true HbA1c values measured later.

Some other modified hemoglobins can be eluted in the same fraction as labile HbA1c. These include carbamylated, acetylated, and aldehyde hemoglobin [17] which are known to be formed in patients with renal failure, aspirin treatment, and excessive alcohol consumption, respectively [18–22]. The first patient had acute renal failure and the second patient was taking aspirin. However, in both cases, the fraction corresponding to labile HbA1c decreased rapidly with improvement in blood glucose levels, suggesting that modified hemoglobin was unlikely to have influenced the results.

Hemoglobin variants have an altered conformation owing to amino acid substitutions caused by genetic mutations [23]. More than 1,400 types of hemoglobin variants have been reported, and the elution time varies widely depending on their chemical characteristics [24]. Therefore, HbA1c values could be falsely low or high, depending on how the chromatogram is disturbed [3, 25, 26]. However, the chance that hemoglobin variants elute simultaneously as labile HbA1c, which elutes earlier than stable HbA1c, is low. Therefore, we performed high-resolution HPLC analysis [27] in the second case to exclude this possibility, but we did not find any obvious hemoglobin variant (data not shown).

In conclusion, HbA1c may not always correctly reflect blood glucose status, especially when glucose levels are extremely high, depending on the measurement modality and pathological conditions. Therefore, when HbA1c is unmeasurable or suspected not to accurately reflect the blood glucose status, it is recommended to check the chromatogram of HPLC, use other HPLC devices or glycated albumin, and retest HbA1c after glucose levels are normalized.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Formal ethics approval was waived for this paper by the ethics committee of Akita University Graduate School of Medicine due to its being a case report.

Informed consent Written informed consent was obtained from the patients for publication of this case report.

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