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【 CASE REPORT 】

Hb Phnom Penh Showing Falsely High or Reasonable HbA1c Values Depending on the Type of High-performance Liquid Chromatography System

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Abstract:
We herein report a 50-year-old Chinese woman with Hb Phnom Penh (α117Phe-Ile-α118Thr) showing high or reasonable HbA1c values depending on the type of high-performance liquid chromatography (HPLC) system. A high HbA1c value of 7.5% (HPLC assay: G9) and a reasonable HbA1c value of 5.2% (assay unknown) were observed. Therefore, the patient was refereed to our hospital; the oral glucose tolerance test showed normal glucose tolerance. The HbA1c values measured by an enzymatic assay, immunoassay, and affinity assay, as well as most HPLC assays were within the reference range, whereas those measured by the Tosoh HPLC systems were high.

Key words: variant hemoglobin, Hb Phnom Penh, HbA1c, high performance liquid chromatography

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Introduction

HbA1c has been widely used as a glycemic control index and a diagnostic index for diabetes mellitus in clinical settings (1). Currently, ion-exchange high-performance liquid chromatography (HPLC) is mainly used for the measurement of HbA1c. However, HbA1c measured by an HPLC assay in standard mode (SM-HPLC) in a patient with variant hemoglobin may not be measured accurately (2). Other techniques, such as affinity chromatography, immunoassays, and enzymatic assays, have also been developed, and the HbA1c levels are currently measured by various methods. While these assays are inferior to an HPLC assay in accuracy, they have the advantage of showing accurate HbA1c values in most patients with variant hemoglobin (2, 3). Consequently, close attention should be paid to the correlation between HbA1c and the plasma glucose level in routine practice.

Since the mobility of variant hemoglobin (HbX0) on SM-HPLC is different from that of normal hemoglobin (HbA0), HbA1c in most cases of variant hemoglobin shows a falsely low value, although a falsely high value may be obtained in some cases (4). We experienced a case of Hb Phnom Penh with a mutation in the α1 chain in the globin gene. We herein report a patient showing falsely high or reasonable HbA1c values, depending on the type of HPLC system.

Case Report

A 50-year-old Chinese woman had a medical history of hypertension, hyperuricemia, chronic kidney disease, and hepatitis B virus as a carrier. This woman visited a physician for proteinuria that had been identified during a medical checkup. A high HbA1c value of 7.5% (58.5 mmol/mol) (SM-HPLC; G9; Tosoh, Tokyo, Japan) was noted, and the patient was instructed to improve her lifestyle. However, the HbA1c value (assay unknown) measured by the patient's primary care physician was within the reference range at

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5.2% (33.3 mmol/mol). The patient was referred to our hospital because of the discrepancy between the HbA1c values measured by different methods. The physical findings of the patient were as follows: height, 154 cm; body weight, 53 kg; body mass index (BMI), 22.4 kg/m^2; and blood pressure, 119/81 mmHg. No significant findings were observed in thoracoabdominal or neurological examinations.

Based on the results of the first medical examination, positive proteinuria and mild impairment of the renal function [serum urea nitrogen, 24.3 mg/dL; serum creatinine, 1.27 mg/dL, estimated glomerular filtration rate (eGFR) 33.1 mL/min/1.73 m^2] were detected (Table 1). Complete blood counts showed no anemia or any other remarkable findings. The HbA1c value measured by the SM-HPLC assay (G9; Tosoh) was high at 7.7% compared to the fasting plasma glucose level of 87 mg/dL. In addition, glycated albumin and 1,5-anhydroglucitol (1,5-AG) values were within the reference range at 14.7% and 16.6 μg/mL, respectively. A 75-g oral glucose tolerance test (OGTT) showed normal glucose tolerance (fasting plasma glucose, 82 mg/dL; 2-hour post-load plasma glucose, 112 mg/dL), and the mean and maximum blood glucose values based on continuous glucose monitoring were 88.3 mg/dL and 160 mg/dL, respectively.

All data on fasting plasma glucose, OGTT, blood glucose tests by continuous glucose monitoring, glycated albumin, and 1,5-AG values were within the reference ranges, while high HbA1c values (7.7%) measured by SM-HPLC (G9) and discrepancies in HbA1c values that might have been due to differences in the measurement methods were observed. Therefore, variant hemoglobin was suspected. A globin gene analysis revealed heterozygous variation with an ATC (isoleucine) codon inserted between 117 and 118 of the α1 chain in the globin gene; Hb Phnom Penh (α117Phe-Ile-α118Thr) was thus diagnosed (Fig. 1).

HbA1c was then measured by various methods using the same sample (Table 2). The HbA1c values measured by an immunoassay, enzymatic assay, and affinity assay were all within the reference range at 5.2% (33.3 mmol/mol), 5.1% (32.2 mmol/mol), and 5.1% (32.2 mmol/mol), respectively. Furthermore, the HbA1c values measured by Arkray SM-HPLC systems (HA-8170 and HA-8181, Arkray, Kyoto, Japan) were also within the reference range at 4.8% (29.0 mmol/mol) for both, showing no abnormality on the chromatograms. The HbA1c value measured by variant mode (VM)-HPLC system (HA-8180T; Arkray) was within the reference range at 4.9% (30.1 mmol/mol). The HbA1c value measured by Tosoh SM-HPLC system (G8) was within the reference range at 5.1% (32.2 mmol/mol), but an abnormal peak between HbA1c and HbA0 was observed on the chromatogram (Fig. 2B). HbA1c values measured using other Tosoh SM-HPLC systems (G7 and G9) were high at 8.0% (63.9 mmol/mol) and 7.1% (54.1 mmol/mol), respectively (Fig. 2A, C). HbA1c measured by Tosoh HPLC showed the same result even when it was measured again, so we confirmed the reproducibility of the findings. The elevation of the baseline values of HbA1c and HbA0 on the chromatogram was observed by both HPLC systems. The HbA1c value measured by Tosoh VM-HPLC system (G8) was within the reference range at 4.7% (32.2 mmol/mol), but the peak of the variant hemoglobin (HbX0) between HbA1c and HbA0 was observed on the chromatogram despite no such findings being noted for the control (Fig. 3).

Ethical approval to perform the globin gene analysis was obtained from the Ethics Committee of Dokkyo Medical University Saitama Medical Center. The patient received a sufficient explanation about the significance and method of this analysis before agreeing to sign the consent form. Furthermore, the patient also agreed with the publication.

Discussion

We experienced a case of Hb Phnom Penh that showed high HbA1c values when measured by Tosoh SM-HPLC systems (G7, G9) despite normal glucose tolerance levels. However, Tosoh G8 (SM-HPLC) and Arkray SM-HPLC systems showed reasonable HbA1c values. This is a rare case of variant hemoglobin showing different HbA1c values depending on the type of HPLC system used.

Hb Phnom Penh was reported by Wajcman for the first time in 1998 (5). Several cases have been reported since then (5-7), but this is the first report from Japan. Most Hb Phnom Penh cases, including the present case, have no hematological abnormalities (5, 7). Only one case complicated with α-thalassemia showed microcytic hypochromic anemia (6). Based on the above, it is considered that Hb Phnom Penh without thalassemia does not show hematological abnormalities.

Chen et al. reported HbA1c in Hb Phnom Penh for the first time (7). Their patient had Hb Phnom Penh with type 2 diabetes mellitus; the HbA1c value measured by the SM-HPLC system (G8) was high at 8.2% (66.1 mmol/mol), while the HbA1c value measured by an affinity assay was 6.2% (44.3 mmol/mol), showing a discrepancy in the HbA1c values between the measurement methods. The chromatogram of G8 resembled the chromatograms of G7 and G 9 in the present study. Based on results of self-monitoring of the blood glucose and fructosamine, the HbA1c values on SM-HPLC were determined to be falsely high. In the present case, the HbA1c values measured by the SM-HPLC systems (G7, G9) were falsely high, while those measured by Tosoh SM-HPLC system (G8) and Arkray SM-HPLC systems were reasonable. The reason why the HbA1c value in this case differed depending on the model of Tosoh HPLC was considered to be because the separation ability differed depending on the model.

In the present case, the HbA1c values measured by some HPLC systems were falsely high, while those measured by most SM-HPLC systems were reasonable. In order to identify reasonable HbA1c values in various types of variant hemoglobin, the following two conditions are necessary: separation between HbX0 and HbA1c shows no overlapping, and mobility is comparable between the two on HPLC. The

Table 1. Laboratory Finding on the First Mediacl Examination.

Parameter	Test value	Unit	Reference range	Parameter	Test value	Unit	Reference range	Parameter	Test value	Unit	Reference range
WBC	5.6×10^2	/μL	3.3-8.6	AST	17	U/L	13-30	BUN	24.3	mEq/L	8-20
RBC	431×10^4	/μL	386-492	ALT	11	U/L	7-23	CRE	1.27	mEq/L	0.46-0.79
Hb	12.4	g/dL	11.6-14.8	ALP	193	U/L	106-322	eGFR	33.1	mL/min/1.73m^2	≤60
Ht	37.2	%	35.1-44.4	LDH	153	U/L	124-222	FPG	87	mg/dL	73-109
MCV	86.3	fl	83.6-98.2	γ-GTP	8	U/L	9-32	HbA1c (HPLC; G9)	7.7	%	4.9-6.0
MCH	28.8	pg	27.5-33.2	T-Bil	0.51	mg/dL	0.4-1.5	GA	14.7	%	11.8-16.0
MCHC	33.3	g/dL	31.7-35.3	D-Bil	0.06	mg/dL	0.0-0.5	1,5-AG	16.6	μg/dL	≤14.0
Plt	20.2×10^4	/μL	15.8-34.8	TP	7.4	g/dL	6.6-8.1	*Urine test*			
Reticulocyte	1.5	%	0.8-2.5	Alb	4	g/dL	4.1-5.1	glucose	(-)		(-)
Serum iron	67	μg/dL	40-188	TG	90	mg/dL	<150	protein	(+)		(-)
TIBC	305	μg/dL	283-441	HDL-C	58	mg/dL	≤40	occult blood	(-)		(-)
UIBC	238	μg/dL	156-369	LDL-C	117	mg/dL	100-159	ketones	(-)		(-)
Ferritin	48.7	ng/mL	3.6-114	Na	142	mEq/L	138-145				
Transferrin	233	mg/dL	200-340	K	4.2	mEq/L	3.6-4.8				
Haptoglobin	122	mg/dL	type 1-1;<83 type 2-1;<65 type 2-2:<25	Cl	108	mEq/L	101-108				

WBC: white blood cell count, RBC: red blood cell count, Hb: hemoglobin, Ht: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, Plt: platelet count, TIBC: total iron binding capacity, UIBC: unsaturated iron binding capacity, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, γ-GTP: γ-glutamyltranspeptidase, T-Bil: total bilirubin, D-Bil: direct bilirubin, TP: total protein, ALB: albumin, TG: Triglyceride, HDL-C: HDL cholesterol, LDL-C: LDL cholesterol, BUN: blood urea nitrogen, CRE: creatinine, eGFR: estimated glemerular filtration rate, FPG: fasting plasma glucose, GA: glycated albumin, 1,5-AG: 1,5-anhydroglucitol

present case is an extremely rare case of variant hemoglobin SM-HPLC assay. that showed reasonable HbA1c values when measured by an Of note, the present patient had renal dysfunction, which

can affect the HbA1c level. She has been diagnosed with chronic glomerulonephritis, and her chronic kidney disease (CKD) stage during these tests was G3bA3 (namely, a moderately impaired renal function and overt proteinuria). However, since a kidney biopsy has never been performed, the pathogenesis of renal dysfunction is unclear. Nonetheless, since renal anemia was not observed, it was unlikely that her renal dysfunction affected the HbA1c level.

We experienced a case of Hb Phnom Penh with normal glucose tolerance that showed falsely high or reasonable HbA1c values, depending on the type of HPLC system. HbA1c is known to not reflect plasma glucose accurately under various conditions, such as in patients with a variant hemoglobin status. Therefore, it is important to assess the glycemic control based on more than just the HbA1c value alone. If there is a possibility that the HbA1c value does not reflect the glycemic control accurately, a comparison of HbA1c values with mean blood glucose levels obtained by continuous glucose monitoring and/or the measurement of glycated albumin and 1,5-anhydroglucitol values (as glycemic control indices that are not affected by variant hemoglobin or anemia) is recommended.

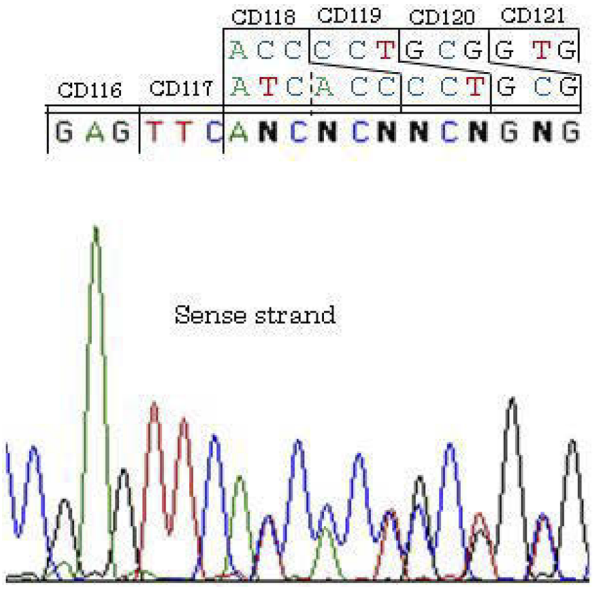

Figure 1. **Results of the globin gene analysis. PCR amplification was performed with DNA extracted from white blood cells for the globin gene analysis with a direct sequencing assay. Nucleotide sequences at between positions 116 and 120 of the α1 chain in this case are shown. The insertion of three nucleotides of ATC (isoleucine) was identified between positions 117 and 118.**

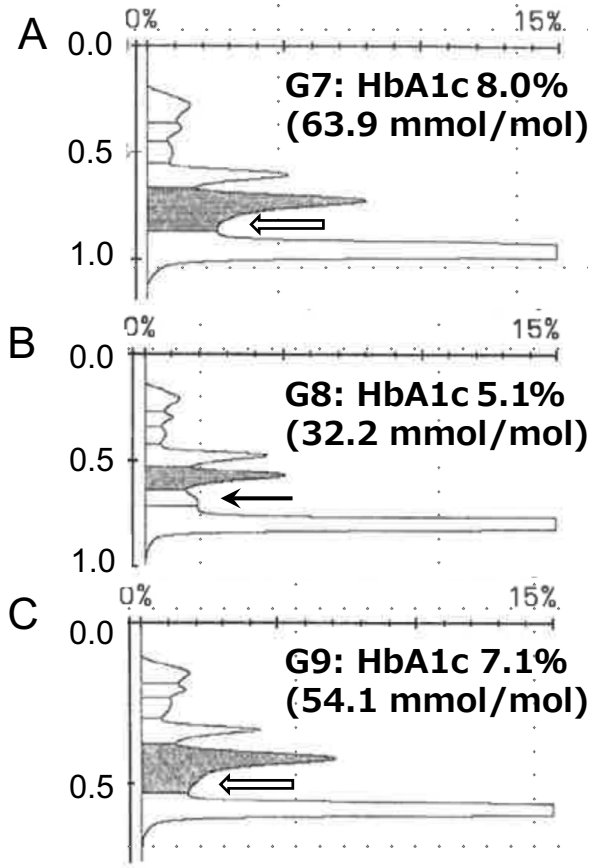

Figure 2. **Chromatograms obtained using the standard mode of Tosoh HPLC systems. Chromatograms obtained using the standard mode of Tosoh HPLC systems G7 (A), G8 (B), and G9 (C) in the present case are shown. The black areas represent HbA1c. White arrows A and C indicate elevated baseline values of HbA1c and HbA0. Arrow B indicates an abnormal peak in which part of the area may have been associated with HbX0.**

Table 2. **HbA1c Values Measured by Various Methods.**

Measurement method (mode)	System type/kit and Company	HbA1c (%)	HbA1c (mmol/mol)
Immunoassay	Banalyst HbA1c, Arkray, Kyoto, Japan	5.2	33.3
Enzymatic assay	CinQ HbA1c, Arkray, Kyoto, Japan	5.1	32.2
Affinity assay	G8 (affinity mode), Tosoh, Tokyo, Japan	5.1	32.2
SM-HPLC assay	G9, Tosoh, Tokyo, Japan	7.1	54.1
SM-HPLC assay	G8, Tosoh, Tokyo, Japan	5.1	32.2
SM-HPLC assay	G7, Tosoh, Tokyo, Japan	8.0	63.9
SM-HPLC assay	HA-8170, Arkray, Kyoto, Japan	4.8	29.0
SM-HPLC assay	HA-8181, Arkray, Kyoto, Japan	4.8	29.0
VM-HPLC assay	G8, Tosoh, Tokyo, Japan	4.8	29.0
VM-HPLC assay	HA-8180T, Arkray, Kyoto, Japan	4.9	30.1

SM: standard mode, VM: variant mode

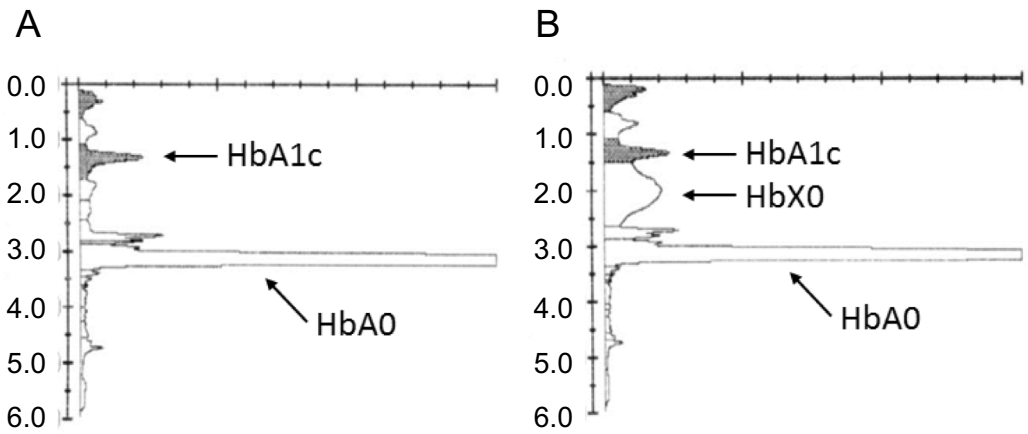

Figure 3. Chromatograms obtained using the variant mode of Tosoh HPLC system. Chromatograms obtained using the variant mode of Tosoh HPLC system (G8) in the present case are shown. A: control sample, and B: sample obtained from this case.

The authors state that they have no Conflict of Interest (COI).

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