A Case of 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 referred 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

Introduction
HbA1c has been widely used as a glycemic control index and a diagnostic index for diabetes mellitus in clinical settings (1). Currently, high-performance liquid chromatography (HPLC) with ion-exchange chromatography 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. 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 Corp., Tokyo, Japan) was noted, and the patient was instructed to improve her lifestyle. However, the HbA1c value (assay unknown) measured by the patient’s
confirmed the reproducibility of the findings. The elevation in the same result even when it was measured again, so we found 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 and 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 alpha-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 G9 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 and 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.
Table 1. Laboratory Finding on the First Medical Examination.

| Parameter | Test value | Unit | Reference range | Parameter | Test value | Unit | Reference range | Parameter | Test value | Unit | Reference range |
|-----------|------------|------|-----------------|-----------|------------|------|-----------------|-----------|------------|------|-----------------|
| WBC       | 5.6×10^3 /μL | 3.3-8.6 | —               | AST       | 17 U/L     | 13-30 | —               | BUN       | 24.3 mEq/L | —    | 3-8.20         |
| RBC       | 4.3×10^4 /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  lmL/min/1.73m² | —    | ≤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 | 0-0.05 | 1.5-AG         | 16.6 μg/dL | ≤14.0 | —           |
| Plt       | 20.2×10^10 /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 | —               | LDL-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 | —               | Cl        | 108 mEq/L  | 101-108 | —               | Type 2-2:≤25 | —        | —             |

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 glomerular filtration rate, FPG: fasting plasma glucose, GA: glycated albumin, 1.5-AG: 1.5-anhydroglucitol
Of note, the present patient had renal dysfunction, which can affect the HbA1c level. She has been diagnosed with chronic glomerulonephritis, and her 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 hemoglobins).
bin or anemia) is recommended.

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

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