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Case Report

Unmeasurable HbA1c result due to hemoglobinopathy

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Abstract

The aim of this study was to show the interference caused by hemoglobinopathy in the measurement of hemoglobin A1c (HbA1c). In our case presentation, we reported two patients whose HbA1c values were unmeasurable when using our laboratory's cation exchange chromatography. We detected HbSβ+ and HbSC variants by remeasuring the samples with another chromatography instrument. Measurement of HbA1c is a commonly performed procedure for the diagnosis of diabetes and for the assessment of blood glucose control in patients with diabetes. However, various hemoglobinopathies, chronic kidney disease, and abnormalities in red cell turnover rate may interfere with HbA1c quantification.

Keywords: Glycated hemoglobin, HbA1c, hemoglobinopathies, HPLC

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Analysis of glycosylated hemoglobin (A1C, hemoglobin A1C, HbA1c) shows the proportion of hemoglobin to which a glucose molecule has been non-enzymatically adjoined. The production of HbA1c is a spontaneous reaction that is dependent on the prevailing concentration of blood glucose. The irreversible production of HbA1c proceeds for approximately 120 days, which is the red blood cell's lifespan. Therefore, HbA1c levels represent the average glucose levels of the previous 2–3 months. HbA1c is the most common test used in chronic glycemic management [1]. As standardized by the Diabetes Control and Complications Trial (DCCT) and approved by the National Glycohemoglobin Standardization Program (NGSP), the measurement levels of ≥6.5% or ≥48 mmol/mol concentration of HbA1c are crucial parts of the diagnostic criterion for Diabetes Mellitus [2].

HbA1c can be measured by enzymatic assay, immunoturbidimetric assay, boronate affinity, ion-exchange High Perfomance Liquid Chorataography (HPLC), or capillary electrophoresis in clinical laboratories [3].

Testing of HbA1c has some advantages over blood glucose level measurement which is affected by calorie intake and involves the necessity of patient preparation, and specific timing. HbA1c measurement also has more preanalytic stability and less variability between days [1].

However, HbA1c cannot be studied in every laboratory due to its rather high cost. Also, inappropriate correlations between HbA1c measurement results and blood glucose levels can be seen in patients with hemoglobinopathies, thalassemia, etc.

It has been stipulated that HPLC, which is considered the gold standard, should be used for HbA1c testing in every laboratory working on HbA1c in the United States [4].

Case Report

In this case report, hemoglobinopathy variants were detected in two cases in which HbA1c test results couldn't be obtained by cation exchange HPLC. The use of fructosamine testing instead of HbA1c in the follow-up will be discussed.

Patients and Methods

Two male patients (aged 29 and 25) were seen by their general practitioner for their routine checkups. Their biochemistry, hemogram, and HbA1c samples were accepted into

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our laboratory. Test results of the patients are presented in Table 1. The patients' HbA1c tests were performed with a cation-exchange HPLC-based Adams A1C HA-8180V (Arkray, Inc., Kyoto, Japan) instrument. HbA1c values were not obtained by our instrument for both patients (Fig. 1). The samples were reevaluated with a cation-exchange HPLC-based Adams A1C HA-8180T (Arkray, Inc., Kyoto, Japan) instrument (Fig. 2). HbSβ+ and HbSC variants were detected from Case 1 and Case 2, respectively.

Table 1. Test results of the patients

Parameters	Results case 1	Results case 2	Reference value
Glucose (mg/dL)	85	72	74–100
Urea (mg/dL)	31		16.6–48.5
Creatinin (mg/dL)	0.96	0.73	0.70–1.20
AST (U/L)	26	30	0–40
ALT (U/L)	14	38	0–41
Iron (ug/dL)	102	65	33–193
UIBC (ug/dL)	184	228	125–345
Calcium (mg/dL)	9.4	9.2	8.6–10
Ferritin (ug/L)	631	136	30–400
TSH (mU/L)	3.87	2.9	0.27–4.2
Free T4 (ng/L)	12.4	12.1	8.9–17.1
WBC (10^3/uL)	9.78	10.48	3.8–10
RBC (10^6/uL)	2.59	4.82	4.3–5.7
Hemoglobin (g/dL)	95	129	132–173
Hematocrit (%)	28.9	35.7	40–52
MCV (fL)	111.7	74	80–95
MCHC (g/dL)	330	362	310–370
Trombocyte (10^3/uL)	400	283	150–400

AST: Aspartate transaminase; ALT: Alanine aminotransferse; UIBC: Unsaturated iron binding capaticy; TSH: Thyroid stimulating hormone; WBC: White blood cell; RBC: Red blood cell; MCV: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

Discussion

The quantity of HbA1c in patient samples is not only affected by blood glucose levels but also influenced by changes in red blood cell lifetime and globin chain structure. Hence, clinical biochemistry, hematological, and analytical methods are all important when commenting on HbA1c [5].

When A1C values are unexpected or incompatible with blood glucose measurement results, a disorder in red cell turnover, hemoglobin variants, and chronic kidney disease should be considered.

Red cell turnover: Incorrectly elevated HbA1c levels can occur when red cell turnover is low. This situation can occur in megaloblastic anemia (related to folate or vitamin B12 deficiency). Conversely, incorrectly low HbA1c levels can occur with fast red cell turnover. This situation can occur in patients

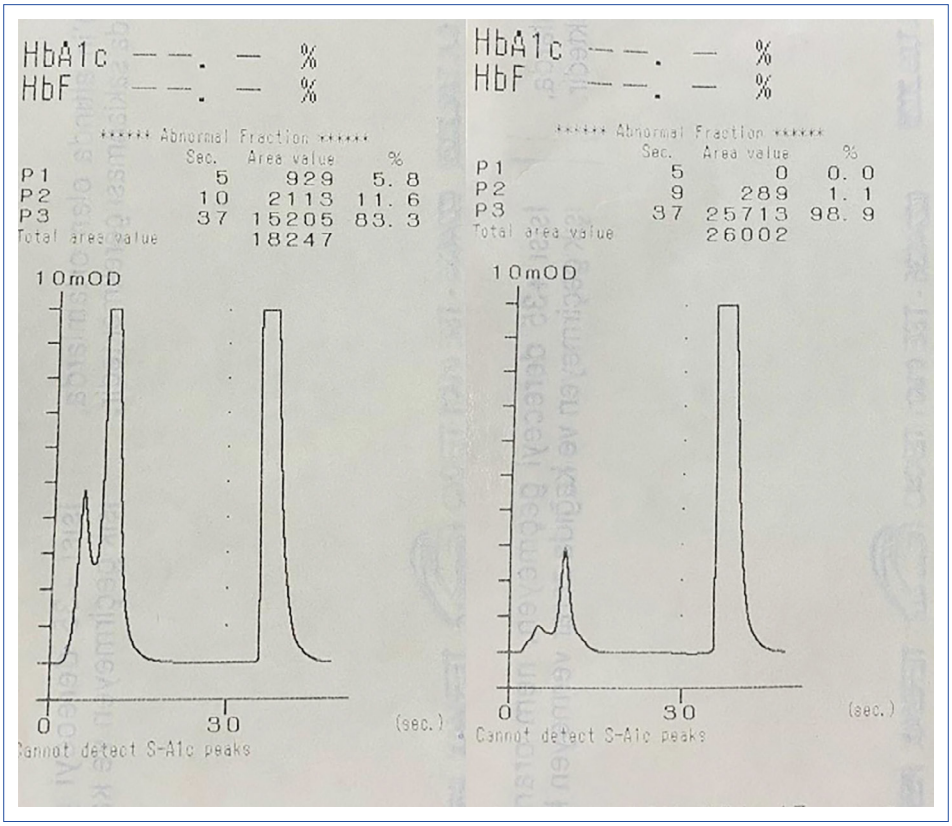

Figure 1. Unmeasurable HbA1c value of cases.
HbA1c: hemoglobin A1C.

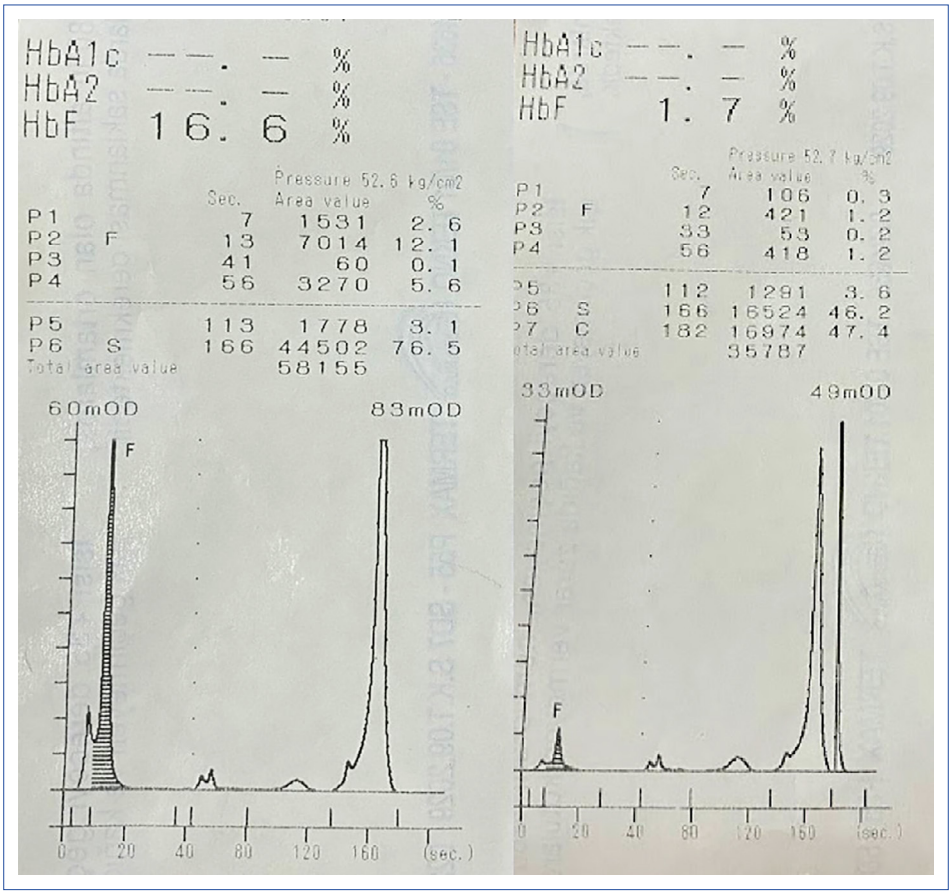

Figure 2. Reevaluated sample of the cases.

with chronic hemolysis (thalassemia, glucose-6-phosphate dehydrogenase deficiency) and patients treated with iron or erythropoietin [6–9].

Hemoglobinopathy: HbA1c levels can be measured high or low in patients with hemoglobin variants depending on the methodology [10].

Chronic kidney disease: HbA1c levels can be altered by advanced chronic kidney disease, hemodialysis, and erythropoietin treatment [11].

All hemoglobin disorders that occur as a result of a genetic disorder are called hemoglobinopathies. Hemoglobinopathies are examined in two main groups; thalassemias and hemoglobin variants. Thalassemia syndromes occur when the defect in the genes causes synthesis disorders without affecting the globin structure. Hemoglobin variants occur when the defect in the genes causes abnormal globin synthesis [12].

Hemoglobin S, associated with sickle cell anemia, is caused by a specific mutation in the beta-globin (HBB) gene (β6 GAG>GTG Glu>Val). This variant can be found in heterozygous (AS, carrier) or homozygous states (SS, patient). The homozygous (SS) form is the most commonly seen form in sickle cell anemia; however, compound heterozygosity of variants of β-thalassemia (Sβ0 and Sβ+) and Hb C (SC) can also be seen in patients showing signs and symptoms of sickle cell anemia [13]. Hemoglobin Sβ-thalassemia occurs with the co-inheritance of HbS and β-thalassemia alleles. HbS and HbA ratios depend on whether the thalassemia allele is β+ or β0 type. Hemoglobin electrophoresis shows 60–90% Hb S, 0–30% Hb A, 1–15% Hb F, and 3.5–6% Hb A2 for Hb Sβ+ thalassemia and 80–95% Hb S, 0% Hb A, 1–15% Hb F, and 3.5–6% Hb A2 for Hb Sβ0 thalassemia [13]. Hemoglobin SC, which has the highest prevalence in West Africa, occurs with the co-inheritance of HbS and HbC alleles. HbSC, which has more target cells and fewer sickle cells in blood smear tests, causes a mild version of sickle cell anemia. Hemoglobin electrophoresis shows 50% Hb S, 0–30% Hb A, 1–7% Hb F, and a normal ratio of HbA2 for HbSC [13]. In our cases, HbA1c values were not measurable by our instrument (Adams A1C HA-8180V), but with another HPLC-based analysis (Adams A1C HA-8180T), HbSβ+ and HbSC were detected in case 1 and case 2, respectively. Genetic analysis was also recommended for the patients to confirm the diagnosis. Macrocytic anemia (Hemoglobin: 95 g/dL and MCV: 111.7 fL) and microcytic anemia (Hemoglobin: 129 g/dL and MCV: 74 fL) were observed in hematology tests from case 1 and case 2, respectively. Tirthankar et al. [14] presented a case whose HbA1c test result was zero by an ion-exchange HPLC method on a Bio-Rad D10 analyzer. They investigated a variant hemoglobin (Hb) by capillary electrophoresis of Hb. They found an elevated peak which was suggestive of HbE disorder. Hegde et al. [15] reported a case whose HbA1c was measured zero by an ion-exchange high-performance liquid

chromatography (HPLC) method on a Bio Rad D10 analyzer. The chromatogram showed a large unknown peak with an area of 82.4% and DNA sequencing confirmed HbD Punjab mutation. Fructosamine should be recommended for clinical use in special populations where HbA1c is less useful. John et al. [16] investigated the clinical use of fructosamine and HbA1c in diabetes mellitus compared to control groups. They found that serum fructosamine should be considered a valid diagnostic marker.

Conclusion

HbA1c results would not be obtained from HbSS, HbSβ+, HbSC, and HbCC patients since these patients would not have any HbA. Alternative test forms such as fructosamine testing should be considered for patients whose HbA is unmeasured in the chromatogram or when HbA1c results are unobtainable by any measurement method.

Informed Consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

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