



Unmeasurable Hemoglobin A1c due to Extreme Hyperglycemia in High-performance Liquid Chromatography Method: A Case Report

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Abstract

BACKGROUND: Hemoglobin A1c (HbA1c), which is one of the important parameters considered when judging the degree of glucose concentrations during the preceding 8–12 weeks, is used as the standard assessment of glycemic control in patients with diabetes. However, some measurement principles may cause errors in HbA1c measurement.

CASE REPORT: A 49-year-old male patient with hyperglycemic hyperosmotic syndrome had an extreme high blood glucose level of 2,161 mg/dL and an HbA1c level that was not measurable due to a large amount of labile HbA1c. Many institutions measure HbA1c by high-performance liquid chromatography (HPLC). We measure HbA1c using ARKRAY HA-8190V (ion-exchange HPLC) and report National Glycohemoglobin Standardization Program (NGSP) (%). HbA1c was not measurable in this case, so we requested glycated albumin measurement as a substitute which revealed an extreme abnormal value of 64.5%. HPLC analysis revealed a large #C peak, which appeared to be labile HbA1c, before the HbA1c peak. The patient was treated with insulin therapy, and the HbA1c was measurable and was 14.1% a day after the glucose level was controlled.

CONCLUSION: The report emphasizes the importance of understanding the limitations of HbA1c measurements in situations of extreme hyperglycemia.

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Introduction

Glycated hemoglobin was derived from the nonenzymatic addition of glucose to amino groups of hemoglobin. Among them, hemoglobin A1c(HbA1c) is a specific glycated hemoglobin where glucose attaches to the N-terminal valine of the hemoglobin β -chain [1]. The hemoglobin structure consists of a tetramer of two pairs of protein molecules: Two α and two non- α globin chains. The α globin chains are classified as HbA1 and HbA2, whereas the non- α globin chains include β , γ and δ . The hemoglobin molecule (HbA) is approximately 97% of normal human adult hemoglobin, which consists of two α chains and two β chains [2]. HbA1c is dependent on the interaction between erythrocyte lifespan and blood glucose concentration and has been proposed as a diagnostic marker for diabetes mellitus. HbA1c is one of the important parameters considered when judging the degree of glucose concentrations during the preceding 8–12 weeks because the average lifespan of erythrocytes is approximately 120 days. Erythrocytes

are constantly produced in the bone marrow and destroyed in the spleen, and only 50% of HbA1c values represent glucose exposure in the immediately preceding 30 days, 40% represent exposure in the immediately preceding 31–90 days, and 10% in the immediately preceding 91–120 days [3], [4].

Currently, many institutions measure HbA1c by high-performance liquid chromatography (HPLC), and we measure HbA1c using the HPLC method (ADAMS A1c HA-8190V, ARKRAY Inc., Japan) and report NGSP (%). HbA1c measurement, particularly HbA1c, is an important tool for monitoring blood glucose levels in individuals with diabetes mellitus. The HbA1c test indicates average blood glucose levels over the past 8–12 weeks and is valuable for screening, diagnosing, and assessing the effectiveness of diabetes management strategies [5], [6]. However, extreme high blood glucose levels may interfere with accurate HbA1c measurement in rare cases. This report presents a case in which HbA1c was not measurable due to extreme high blood glucose levels. We discuss the potential reasons and the implications for clinical examination in such cases.

Case Report

A 49-year-old male patient was referred to the Emergency Department of Fujita Health University Hospital (Aichi, Japan) because of decreased appetite and consciousness disorder after taking medication for manic depression. He had a history of manic-depressive illness and hyperlipidemia but with no diabetes. He had been diagnosed with manic-depressive illness about two decades ago and was consuming 200 mg of lithium carbonate, a medication utilized for the management of bipolar disorder, thrice daily following each meal. He experienced appetite loss approximately 1 week before visiting the emergency department and drank 1 L of sports drinks and 4 L of mineral water daily. He did not sleep through the night and drank water although the details of what he drank on the day before transport are unknown, and he also did not eat at all on the day of transport. His response became sluggish after taking oral medication for manic depression during the night, and his wife called for an ambulance. He was transported to our hospital for emergency care. Biochemistry data demonstrated high glucose (2161 mg/dL), plasma osmolarity (400 mOsm/kg H₂O), and creatinine (3.08 mg/dL), blood gas analysis showed acidosis (pH: 7.119), and base excess (-18.0 mEq/L) and HbA1c was impossible to measure, although blood count data showed no abnormalities. Hence, we requested glycated albumin (GA) measurement as a substitute, which revealed an extreme high value of 64.5% (Table 1). The patient was admitted to our hospital and treated by endocrinologists after the hyperglycemia hyperosmotic syndrome (HHS) and diabetic ketoacidosis (DKA) diagnosis. The patient was managed at the endocrinology and metabolism department with continuous insulin and supplemental fluids, and blood was drawn the next day with Glu at 318 mg/dL and measurable HbA1c at 14.1% (Figure 1). The unmeasurable samples were sent to ARKRAY Kyoto Laboratory for analysis a few days later. HPLC analysis detected no peak that appeared to be mutated hemoglobin. However, a large #C peak, appearing to be labile HbA1c, was detected before the HbA1c peak (Figure 2). Furthermore, unmeasurable samples could be measured by another HPLC instrument ARKRAY HA-8180T and the enzymatic method, 12.7%

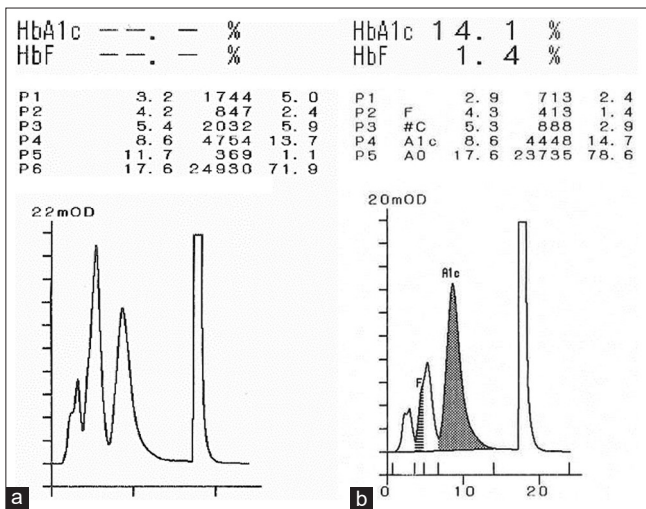


Figure 1: (a) Chromatogram with abnormal peak fractions observed in the HPLC method for hemoglobin A1c measurement. (b) Chromatogram of HbA1c measurement in HPLC method that became measurable the next day after insulin therapy administration in the endocrinology department

by ARKRAY HA-8180T, and 14.8% by the enzymatic method.

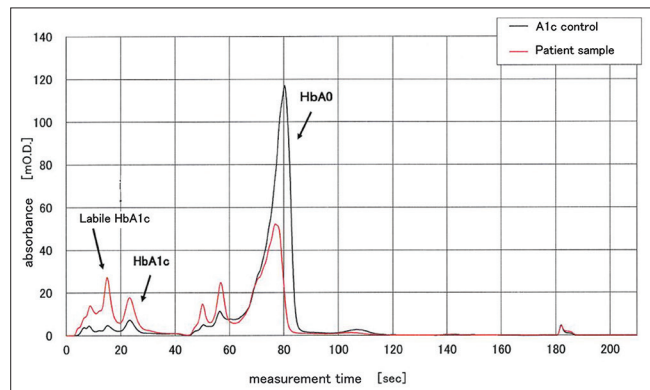


Figure 2: ARKRAY Kyoto Laboratory analysis by HPLC method

Discussion

In 1988, the American Diabetes Association (ADA) recommended glycated hemoglobin or HbA1c

Table 1: Laboratory data

Complete blood count	Value	Biochemistry	Value	Coagulation	Value
WB	16,000/ μ L	TP	5.1 g/dL	PT	99%
Stab	2%	Alb	3.2 g/dL	PT: RATIO	1.00
Seg	86%	AST	13 u/L	PT: INR	1.00
Eo	0%	ALT	33 u/L	PT: Sec	12.8 s
Lym	6%	γ -GT	59 u/L	APTT	25.1 s
Mono	6%	LD	170 u/L	Fib	249 mg/dL
RBC	412 \times 10 ⁴ / μ L	CRP	1.14 mg/dL	D-dimer	1.1 μ g/mL
Hb	12.8 g/dL	Cre	3.08 mg/dL		
Hct	41.4%	Na	111 mmol/L	Blood gas	
Plt	29.4 \times 10 ⁴ / μ L	K	6.4 mmol/L	pH	7.119
MCV	100 fL	Cl	74 mmol/L	Base excess	-18.0 mEq/L
MCH	31.1 pg	Ca	8.7 mg/dL		
MCHC	30.9%	Glucose	2,161 mg/dL	TSH	0.620 μ IU/mL
		HbA1c (NGSP)	unmeasurable	FreeT3	1.350 pg/mL
		GA	64.5%	FreeT4	0.892 ng/dL
		Osm	400 mOsm/kgH ₂ O	GAD	<5.0

Hb: Hemoglobin, CRP: C-reactive protein, RBC: Red blood cell, Hct: Hematocrit, Plt: Platelet, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration.

for standard glyceamic control assessment in patients with diabetes [7]. At first, HbA1c was not recommended for diagnosing diabetes; however, assay improvements confirmed ADA in 2010 as a diagnostic criterion for diabetes at a cut-off of $\geq 6.5\%$, pre-diabetes between 5.7% and 6.4%, and normal at $< 5.7\%$ [8]. Thus, HbA1c is a major biomarker used as a gold standard for assessing long-term glyceamic control (2–3 months) in patients with diabetes and has been reported to correlate with the development of complications such as cardiovascular diseases and cognitive problems [9], [10]. Proteins are frequently glycated during various enzymatic reactions under physiologically favorable conditions. However, glycation occurs by the attachment of glucose to the N-terminal valine of the hemoglobin β -chain without enzymatic attachment in the case of hemoglobin. Labile HbA1c (Schiff base) formed by such non-enzymatic attachment undergoes slow and almost irreversible glycation by Amadori rearrangement, and the final product is the best-known HbA1c (stable ketoamine) (Figure 3) [11], [12], [13]. While HbA1c is a major biomarker used as a gold standard for assessing long-term glyceamic control, it is imperative to acknowledge certain aspect concerning the interconnection between the lifespan of erythrocytes and HbA1c. HbA1c increases in conditions that increase the average lifespan of erythrocytes, such as Vitamin B₁₂ deficiency, iron deficiency and erythropoietin deficiency in renal failure [14]. Conversely, hemolytic anemia and increased hemolysis rates due to drugs such as antiretrovirals may cause a decrease in HbA1c [15], [16].

The reason for the unmeasurable HbA1c was the #C peak that exceeded 4% and “abnormally high #C” in this case. Labile HbA1c cannot be separated from HbA1c in HbA1c measurements using the HPLC method, which may result in a pre-analytical error, although the origin of #C detected in this case is the previously mentioned labile HbA1c. “Abnormally high #C” was caused by a Glu of 2161 mg/dL, indicating the presence of a large amount of labile HbA1c. The unmeasurable sample became measurable after being submitted to ARKRAY Kyoto Laboratory because the labile HbA1c changed to a stable form over time and

HbA1c became measurable as the #C peak decreased. Labile HbA1c directly depends on the blood glucose concentration and can be considerably increased after drinking a lot of sugar and ingesting carbohydrates. Conversely, glucose attached to labile HbA1c may spontaneously dissociate over time when the surrounding glucose concentration is lowered by insulin therapy and other procedures. Additionally, a case with an unmeasurable HbA1c due to extreme hyperglycemia, similar to the present case, has been reported [17]. The blood glucose concentration was 1464 mg/dL, GA was 20.6%, and blood gas analysis showed severe acidosis (pH: 6.968) and base excess (-28.8 mEq/L) in that case. The case was finally diagnosed with diabetic ketoacidosis based on these findings. HbA1c was retested the next day by the HPLC method after changing to insulin abortive therapy, and HbA1c was 7.1% (Plasma glucose was 212 mg/dL) [17]. GA, which was measured this time as a substitute for HbA1c, is one of the validated tests as an alternative glyceamic marker, produced through the glucose to albumin in a nonenzymatic reaction. Presently, GA can be measured by enzymatic assays in automated analyzers designed. GA, which is a glycoprotein produced by the nonenzymatic reaction of glucose and albumin, is one of the effective tests as an alternative blood glucose marker. GA is hemoglobin/erythrocyte independent and reflects the average glucose concentration over the preceding 2–3 weeks, rather than 2–3 months with HbA1c [18], [19]. The relationship between GA and HbA1c was reported as $GA = 2.26 + 2.74 \text{ HbA1c}$, indicating that GA is approximately 3 times higher than HbA1c [20].

The GA and HbA1c of this case were 64.5% and 14.1%, respectively, suggesting a hyperglycemic state for 1–2 weeks immediately before transport. Analysis at ARKRAY Kyoto Laboratory revealed that the HbA1c by the enzymatic method was 14.8% and the HPLC method was similar at 12.7%, making mutant hemoglobin unlikely. A possible cause of the unmeasurable HbA1c is an increased #C peak due to an increased labile HbA1c. Hyperglycemic crises, such as in this case, HHS and DKA in the early stages, remain highly life-threatening if treated incorrectly; therefore,

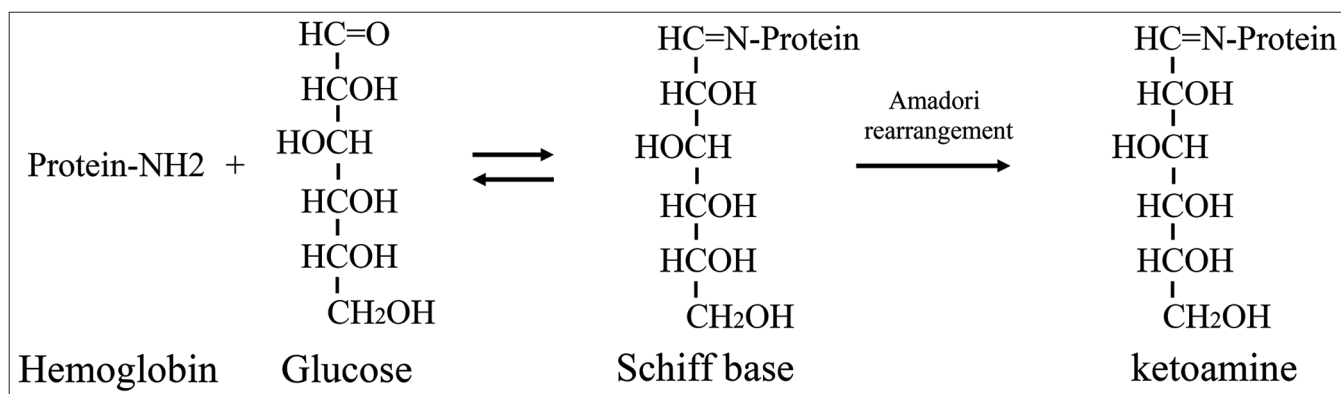


Figure 3: Formation of glycated hemoglobin from the binding of glucose to hemoglobin

sufficient knowledge should be acquired, and accurate and prompt treatment should be practiced [21], [22].

Conclusion

Unmeasurable HbA1c, such as in this case, should be substituted with a possible GA that can be diluted and reported to the clinician. The principle of measurement of each test item should be understood and reported to the clinician as useful criteria for diagnosis as a person involved in clinical examination.

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