HbA1c = 0%? Challenging the Gold Standard: The Unreliability of HbA1c in Diabetes Patients with HbE/β-Thalassemia

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Abstract

Glycated haemoglobin (HbA1c) is widely regarded as the gold standard for monitoring long-term glycaemic control in diabetes mellitus. However, its reliability is significantly compromised in individuals with haemoglobinopathies such as haemoglobin E (HbE) and β-thalassaemia. We report a rare case of a 45-year-old woman with type 2 diabetes mellitus and confirmed HbE/β-thalassemia who presented with an undetectable HbA1c level (0.0%) using ion-exchange high-performance liquid chromatography (IE-HPLC), despite stable fasting and postprandial glucose levels. Post-dilution HPLC analysis revealed a dominant abnormal peak (89.5%), later confirmed as HbE through hemoglobin electrophoresis. Longitudinal data from 2016 to 2024 showed erratic HbA1c trends across analytic platforms. Earlier measurements using turbidimetric immunoassay (TINIA) yielded detectable but likely inaccurate HbA1c values due to cross-reactivity. In contrast, calculated HbA1c values derived from validated glucose-based formulas may be more consistent with the patient's glycaemic status. This case highlights the diagnostic challenges posed by structural hemoglobinopathies, which interfere with glycation kinetics and chromatographic detection, leading to clinically misleading HbA1c results. Although alternative biomarkers such as glycated albumin or fructosamine may improve diagnostic accuracy, these were not available in our setting, reflecting a common limitation in resource-constrained environments. Clinicians must be aware of these pitfalls and adopt individualized monitoring strategies in regions with high hemoglobinopathy prevalence, such as Southeast Asia. Integrating clinical judgment with alternative or calculated glycaemic markers may help ensure appropriate diabetes management in this unique patient population.

Keywords: Glycated Hemoglobin A; Diabetes Mellitus; Hemoglobinopathies; Beta-Thalassemia; High-Performance Liquid Chromatography

Introduction

Glycated haemoglobin (HbA1c) is widely regarded as the gold standard for monitoring long-term glycaemic control in diabetes mellitus; however, its reliability is compromised in individuals with haemoglobinopathies, such as haemoglobin E (HbE) and β-thalassemia.[1–6] In regions where HbE is prevalent, particularly Southeast Asia, patients with diabetes and concurrent HbE/β-thalassaemia often exhibit significant discrepancies between HbA1c values and actual glycaemic status.[7–10] This case highlights an extreme scenario in which a diabetic patient with HbE/β-thalassaemia presented with an undetectable HbA1c level using ion-exchange high-performance liquid chromatography (IE-HPLC), despite stable blood glucose levels. Longitudinal data spanning eight years demonstrated erratic HbA1C trends, further emphasizing the limitations of this biomarker

in this specific population. Additionally, calculated HbA1c values using established formulas provided markedly different results, reinforcing the unreliability of direct HbA1c measurements.[11–13] This case shows how important it is to quickly reevaluate the clinical usefulness of HbA1c in people with hemoglobinopathies.

Case Report

A 45-year-old woman presented to Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, for routine diabetes mellitus management, with no current complaints. She had no history of fever, digestive disorders, malignancy, other metabolic diseases, or autoimmune conditions. The patient had a family history of diabetes mellitus and had been diagnosed with the condition 10 years prior. A physical examination revealed no abnormalities. The physician referred her for laboratory tests, including fasting blood glucose (FBG), 2-hour postprandial glucose (2hPPG), HbA1c, lipid profile, and routine haematology examinations.

The results are shown in Table 1. HbA1c, measured using IE-HPLC (Bio-Rad D-10), was undetectable (0.0%) (Figure 2). Even after sample dilution, the HbA1c remained undetectable. However, post-dilution analysis revealed a variant-window (89.5%) in the chromatogram (Figure 3). A peripheral blood smear showed a hypochromic microcytic morphology with target cells (Figure 1), prompting further haemoglobin electrophoresis. The electrophoresis results confirmed the presence of beta-thalassaemia with HbE.

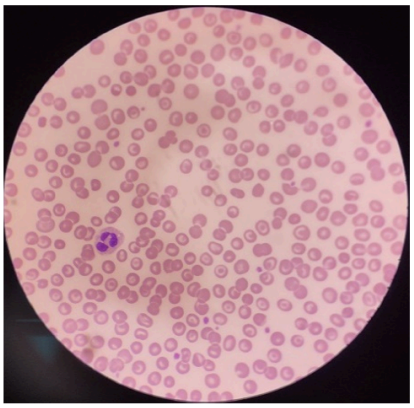


Figure 1: Blood smear evaluation.

Table 1: Laboratory Result.

Parameter	**Result**	**Reference range**
HbA1c	0	3,9 – 6,1
FBG	107	<126
2hPPG	119	Normal : <140
		DM : >200
Haemoglobin	12.5	11.0 – 14.7
MCV	60.5	86.7 – 102.3
MCH	20.4	27.1 – 32.4
MCHC	33.7	29.7 – 33.1
Erythrocytes	6.13	3.69 – 5.46
Total cholesterol	266	< 200
HDL	53	40 – 60

LDL	201	< 100
Uric acid	4.7	2.6 – 6.0

Subsequently, we reviewed the patient's glucose and HbA1c data from her monitoring at our hospital. Our findings revealed inconsistencies between FBG and HbA1c levels. We also calculated HbA1c using the formula: HbA1c Calculated-1 = 2.6 + (0.03 × FBG), Calculated-2 = (0.0357 × fasting blood glucose)+1.915 with the results presented in Table 2.

Table 2. History of HbA1c, FBG and 2hPPG examination (2016 – 2024)

	2016	**2017**	**2018**	**2018**	**2018**	**2019**	**2024**
HbA1c	7.7	7.5	7.4	7.3	6.8	6.5	**0**
FBG	175	118	136	159	186	105	107
2hPPG	324	240	208	277	-	266	119
HbA1c Calculated-1[12]	7.67	6.03	6.55	7.21	7.98	5.66	5.72
HbA1c Calculated-2[13]	8.16	6.13	6.77	7.59	8.55	5.66	5.73
Method	TINIA	TINIA	TINIA	TINIA	TINIA	TINIA	IE-HPLC

***** *TINIA : Turbidimetric Inhibition Immunoassay*

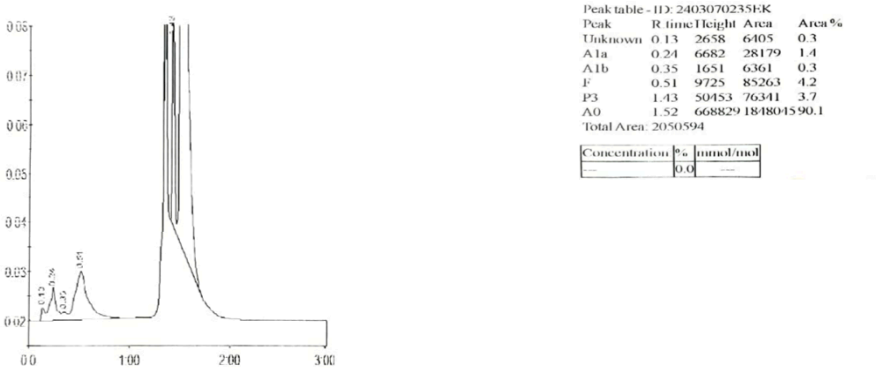
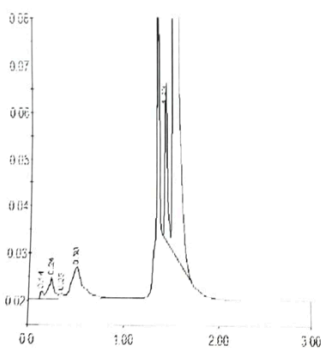


Figure 2: HPLC pre-dilution graph. No variant-window was found

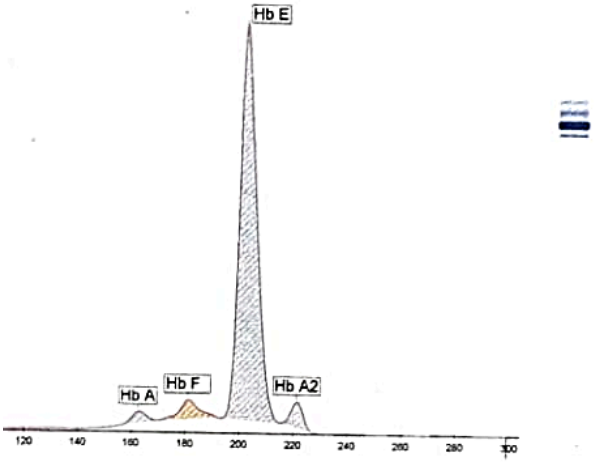


Peak table - ID: 2403070235EK

Peak	R.time	Height	Area	Area %
Unknown	0.14	1538	3884	0.3
A1a	0.24	4514	18440	1.4
A1b	0.35	1085	3742	0.3
F	0.50	6661	54836	4.1
A0	1.43	33802	60861	4.5
Variant-Window	1.53	478292	1207005	89.5
Total Area:		1348767		

Concentration	%	mmol/mol
	0.0	

Figure 3: HPLC post-dilution graph. A variant-window appears with an area of 89.5%



Name	%
Hb A	2.0
Hb F	4.8
Hb E	89.3
Hb A2	3.9

Figure 4: Haemoglobin Electrophoresis Graph; Hemoglobinopathy E and beta-thalassaemia

Discussion

The accuracy of HbA1c as a gold standard for glycaemic control assessment is challenged in patients with hemoglobinopathies, particularly HbE/β-thalassemia.[1,2,5,14] In this case, the complete absence of detectable HbA1c using IE-HPLC highlights the severe limitations of this method in patients with structural haemoglobin abnormalities. We collected a substantial amount of HbA1c data was collected from 2016 to 2024. Previously, examinations were performed using the Turbidimetric Inhibition Immunoassay (TINIA) method on the Dimension RXL analyzer, and no reading failures were observed (Table 2). These values, however, may have been falsely elevated due to antibody cross-reactivity with variant hemoglobins, a known limitation of immunoassay-based HbA1c methods.[6,7,14] In late 2019, our hospital laboratory transitioned to IE-HPLC (the Bio-Rad D-10 system). The complete absence of detectable HbA1c using this method (0.0%) highlights the severe limitations of chromatographic techniques in patients with structural haemoglobin abnormalities. This was further confirmed by post-dilution chromatographic analysis, which revealed an abnormal peak (89.5%), suggesting interference from the haemoglobin variant.[7,15] The Hb variant was subsequently confirmed by hemoglobin electrophoresis, which identified HbE (89.3%). HbE is known to alter haemoglobin glycation kinetics and interfere with separation in chromatography, leading to suppression or displacement of the HbA1c detection window and unreliable HbA1c readings.[2,5,14] The observed discrepancy between directly measured and

calculated HbA1c (based on fasting glucose) underscores the need for clinicians to interpret HbA1c results cautiously and consider alternative monitoring approaches in populations with prevalent hemoglobinopathies.[11,12]

A key finding in this case was the extensive longitudinal HbA1c data spanning eight years, which revealed erratic fluctuations and an eventual undetectable value despite stable fasting and postprandial glucose levels.[2,8] Such inconsistencies indicate that HbA1c alone may not be a reliable glycaemic marker in these patients. Calculated HbA1c, derived using validated formulas for patients with diabetes mellitus, may provide values that align more closely with the patient's actual glycaemic state; however, no studies have yet been conducted in hemoglobinopathies.[11–13] Incorporating calculated HbA1c with direct measurement of HbA1c indicated the measurement is unreliable.[11–13] Ideally, alternative markers such as glycated albumin or fructosamine would provide a more comprehensive glycaemic profile in patients with hemoglobinopathies.[2,13,14] Unfortunately, in this case, this testing was not available at our laboratory due to logistical and resource constraints.

Given the widespread reliance on HbA1c in diabetes management, it is crucial to recognize its limitations in patients with hemoglobinopathies. The World Health Organization (WHO) and the American Diabetes Association (ADA) recommend HbA1c as a standard parameter for both diagnosis and long-term glycaemic monitoring. However, this approach presents unique challenges in regions with a high prevalence of hemoglobin variants, such as HbE.[1,14,15]

Conclusion

This case report underscores a critical limitation in the use of HbA1c as a universal biomarker for glycaemic control, particularly in patients with structural hemoglobinopathies such as HbE/β-thalassemia. The complete absence of detectable HbA1c despite normoglycemic profiles illustrates how conventional analytical platforms like IE-HPLC may yield misleading results due to variant interference. Retrospective analysis further revealed erratic HbA1c trends inconsistent with glucose levels, emphasizing the unreliability of direct HbA1c measurement in this context. This report challenges the current gold standard and calls for greater clinical awareness, especially in high-prevalence regions, to adopt personalized monitoring strategies integrating clinical judgment, alternative biomarkers, and calculated glycaemic indices. Broader access to reliable alternatives like glycated albumin or fructosamine, along with further validation studies, is essential to ensure safe and accurate diabetes management in hemoglobinopathy-affected populations.

Disclosure

The authors declared no conflicts of interest. Written consent was obtained from the patient.

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