CASE REPORT

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Challenges in Glycemic Monitoring: Undetectable HbA1c Level in an Asymptomatic Patient With Hemoglobin E and Beta-Thalassemia Minor: A Case Report

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Abstract

Glycated hemoglobin (HbA1c) is a laboratory examination recommended by the American Diabetes Association to monitor blood glucose levels in patients with diabetes mellitus. This gold standard method for testing HbA1c uses high-performance liquid chromatography (HPLC). One advantage of HbA1c testing with HPLC is the ability to detect Hb variants or hemoglobinopathies, although HbA1c results may become irrelevant in such conditions. A 49-year-old female patient came to the Ophthalmology Outpatient Clinic of Dr. Soetomo Hospital due to visual impairment in both eyes and plans for cryotherapy surgery. In preparation for the surgery, laboratory tests were performed with results of haemoglobin 12.1 g/dL, mean corpuscular volume 58.9 fL, mean corpuscular haemoglobin 19.3 pg, leukocytes 13,370/uL, platelets 399,000/uL and random blood glucose 127 mg/dL; however, HbA1c was not detected. In the HbA1c test, a 93.7% window variant was obtained. Based on blood smear evaluation, there was hypochromic microcytic anisopoikilocytosis with target cells. The electrophoresis results revealed HbA 0.7%, HbF 0.6%, HbE 94.9% and HbA2 4.7%, indicating the presence of beta thalassemia with hemoglobinopathy E. The diagnosis of hemoglobinopathy E is based on a complete blood count, peripheral blood smear and haemoglobin electrophoresis. HbA1c examination by HPLC can yield undetectable HbA1c levels in hemoglobinopathies. Monitoring blood glucose levels in such cases is also irrelevant when using HbA1c, because red blood cells have a shorter lifespan in the presence of Hb variants. Glucose monitoring in this condition can be done by periodic glucose examination, fructosamine, or glycated albumin. The HbA1c test cannot be used as a marker of glucose control in patients with hemoglobinopathy E.

Key words: hemoglobinopathy, HbA1c, HPLC, electrophoresis

INTRODUCTION

Glycated hemoglobin or hemoglobin A1c (HbA1c) is a hemoglobin fraction commonly used to evaluate long-term glycemic control and the risk of chronic complications in patients with diabetes mellitus. The American Diabetes Association (ADA) recommends HbA1c as one of the diagnostic criteria related to diabetes mellitus because it can reflect glucose bound to hemoglobin throughout the life of the erythrocyte (approximately 120 days), so that it can indirectly reflect clinical changes related to glucose exposure during this period, although factors that can affect hemoglobin concentration or erythrocyte turnover can also influence it.[1]

Several methods are known for HbA1c testing, including immunoassay, boronate affinity, enzymatic, capillary electrophoresis and ion-exchange high-performance liquid chromatography (HPLC). The latter two methods offer additional advantages beyond measuring HbA1c, as they can also detect various hemoglobin types and Hb variants. HPLC testing itself is known to have high sensitivity and specificity and can be used as an alternative to electrophoresis in identifying and measuring normal and abnormal hemoglobin fractions. The retention time obtained from HPLC testing can provide information about hemoglobin variants in a sample, both in measuring HbA1c, HbA2, and HbF levels. This allows for monitoring therapy and the progression of diabetes mellitus, as well as detecting recessively inherited hemoglobinopathies such as thalassemia and hemoglobinopathy E.[2,3]

Located in the thalassemia belt region, the prevalence of thalassemia carriers in Indonesia, according to WHO, is around 6-10% of the population, so it is estimated that there are 6-10 thalassemia carriers out of 100 residents.[4]

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More specifically, local estimates indicate that approximately 3.0–10.0% of the Indonesian population carries the beta-thalassemia trait and 2.6–11.0% carry the alpha-thalassemia trait, with around 2,500 infants born with beta-thalassemia major each year.[5] Hemoglobin E is also among the most common structural hemoglobin variants in Southeast Asia and is frequently co-inherited with beta-thalassemia.[5] Importantly, Indonesia's national newborn screening program is currently mandated only for congenital hypothyroidism and does not include hemoglobinopathies; consequently, individuals with beta-thalassemia minor or hemoglobin E commonly remain undetected until incidental laboratory testing later in life.[6] This makes hemoglobinopathy a condition that is not rarely encountered; however, due to the absence of significant clinical symptoms in sufferers, especially in carriers, the choice of examination method can be very decisive.

CASE

A 49-year-old female presented to the Eye Clinic at Dr. Soetomo General Hospital complaining of impaired vision in both eyes. She was reported to have blurred vision in her right eye and undetectable vision in her left eye. The patient did not complain of eye pain, fever, or impaired eye movement. She had no history of malignancy or metabolic disease, but had a history of hypertension and had not been taking her medication regularly. A physical examination revealed decreased visual acuity in both eyes and a leucocele over the entire surface of the left cornea. The attending physician diagnosed the patient with phthisis bulbi and left corneal leucocele and planned cryotherapy (CRI).

Prior to the surgical procedure, routine laboratory testing revealed a random blood glucose level of 127 mg/dL (N: <200 mg/dL) and an undetectable HbA1c result. HbA1c measurement was performed using the Bio-Rad D-10 high-performance liquid chromatography (HPLC) system. The chromatogram showed no detectable HbA1c peak at the expected retention time of approximately 2.0 to 2.2 minutes, with an abnormal chromatographic disturbance observed near this region. No variant window was initially identified (Figure 1). Because of this unexpected finding, the sample was diluted and reanalyzed. The repeated chromatogram again demonstrated the absence of the HbA1c peak at the expected retention time, while a prominent variant window peak occupying 93.7% of the total chromatogram area became evident, indicating the presence of a hemoglobin variant (Figure 2).

Other laboratory findings from the complete blood count are summarized in Table 1, demonstrating hemoglobin 12.1 g/dL, hematocrit 36.9%, erythrocytes 6,270,000/uL, mean corpuscular volume (MCV) 58.9 fL, mean corpuscular hemoglobin (MCH) 19.3 pg, leukocytes 13,370/uL, and platelets 399,000/uL (Figure 3). Based on the complete blood count, the patient's Mentzer index was 9.4, suggesting a possibility of thalassemia, as the number

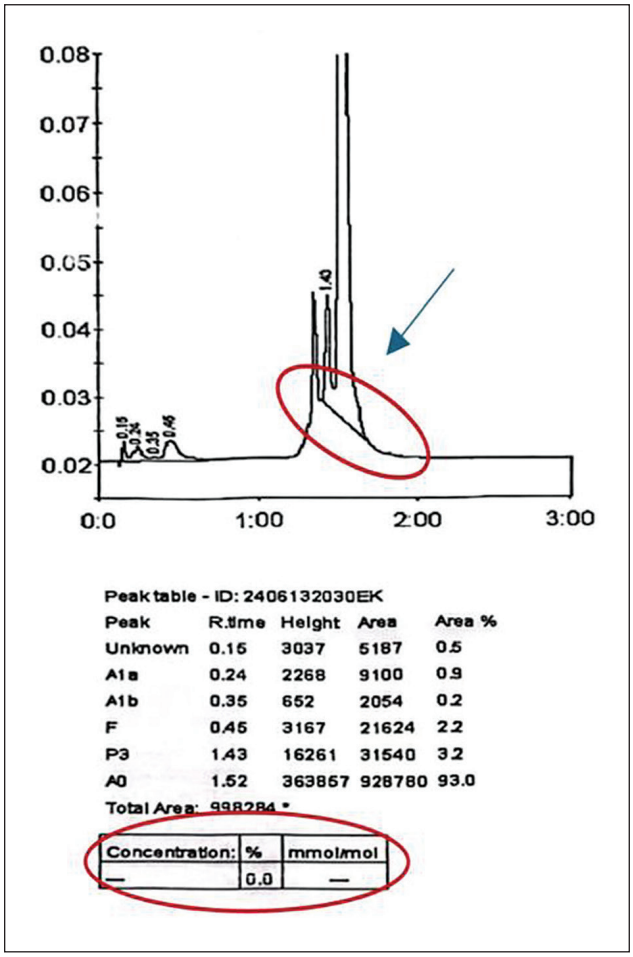

Figure 1. Pre-dilution HbA1c chromatogram using the Bio-Rad D-10 HPLC system. An undetectable HbA1c value is shown. The HbA1c peak, normally expected at a retention time of 0.79–1.01 seconds, was not identifiable in this chromatogram, likely due to masking by an extremely elevated hemoglobin variant that disrupted the normal elution pattern. The transverse line is visible, which may serve as an indirect diagnostic indicator of a high-concentration abnormal hemoglobin fraction. No variant window was detected.

of erythrocytes remained high despite a small size of the red blood cells. Peripheral blood smear examination was then carried out and revealed microcytic hypochromic erythrocytes with (+) target cells suspicious for thalassemia (Figure 4). Furthermore, electrophoresis was performed, and the results obtained were HbA 0.5% (N: 96.8-97.5%), HbF 0.6% (N: 0.0%), HbE 94.4% (N: not detected), HbA2 4.5% (N: 2.4-2.9%). Based on these results, a decrease in the HbA fraction and an increase in the HbF, HbE and HbA2 fractions were observed, suggesting beta thalassemia with hemoglobinopathy E (Figure 5).

Asymptomatic patients with no history of the disease in their family are advised to have a genetic consultation for screening for genetic disorders that may be transmitted through them to their offspring.

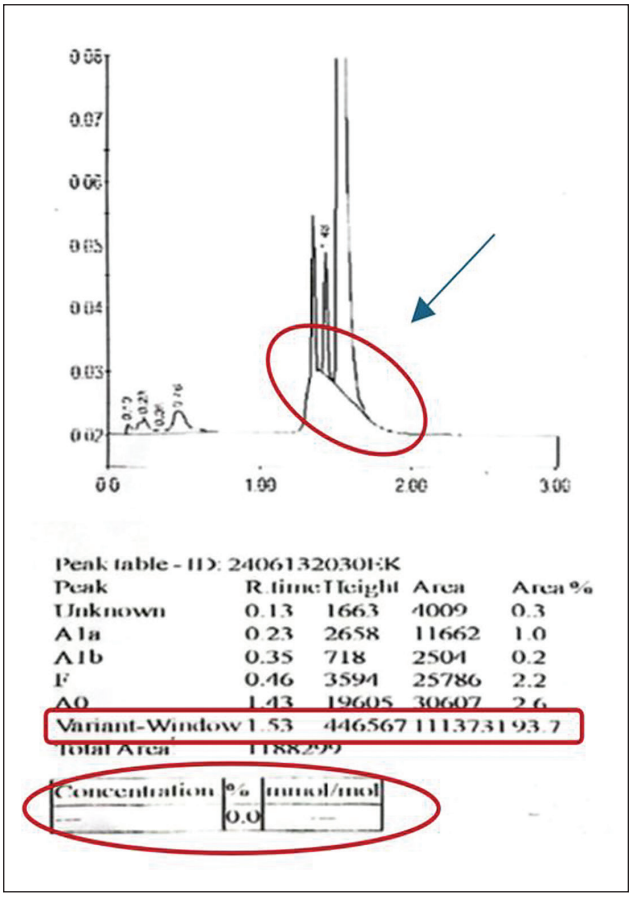

Figure 2. Post-dilution HbA1c chromatogram using the Bio-Rad D-10 HPLC system. An undetectable HbA1c value is still present after sample dilution. The HbA1c peak remains undetectable even sampel dilution. The transverse line is still visible on the chromatogram, and a variant window is present with a total area of 93.7%, consistent with a highly elevated hemoglobin variant.

Table 1. Complete blood count results

Parameter	Result	Unit	Reference range
Hemoglobin (Hb)	12.1	g/dL	Male: 13.3–16.6 Female: 11.0–14.7
Red Blood Cells (RBC)	6.27	$\times 10^6$/uL	3.69–5.46
Hematocrit (HCT)	36.9	%	Male: 41.3–52.1 Female: 35.2–46.7
Mean Corpuscular Volume (MCV)	58.9	fL	86.7–102.3
Mean Corpuscular Hemoglobin (MCH)	19.3	pg	27.1–32.4
Mean Corpuscular Hemoglobin Concentration (MCHC)	32.8	g/dL	29.7–33.1
RDW-SD	31.8	fL	41.2–53.6
RDW-CV	17.7	%	12.2–14.8
White Blood Cells (WBC)	13.37	$\times 10^3$/uL	3.37–10.0
Neutrophils	66.3	%	39.8–70.5
Lymphocytes	23.1	%	23.1–49.9
Monocytes	5.8	%	4.3–10.0
Eosinophils	4.4	%	0.6–5.4
Basophils	0.4	%	0.3–1.4
Platelets (PLT)	399	$\times 10^3$/uL	150–450
Mean Platelet Volume (MPV)	9.7	fL	9.2–12.0
Platelet Distribution Width (PDW)	11.7	fL	9.6–15.2
Plateletcrit (PCT)	0.39	%	0.19–0.39

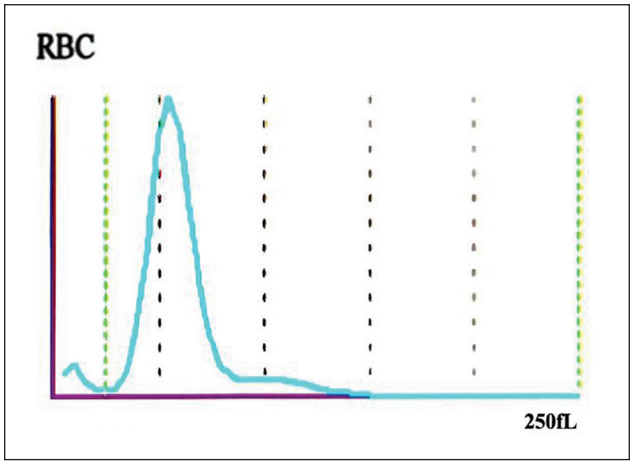

Figure 3. Red blood cell (RBC) histogram showing a left-ward shift consistent with microcytic red blood cells.

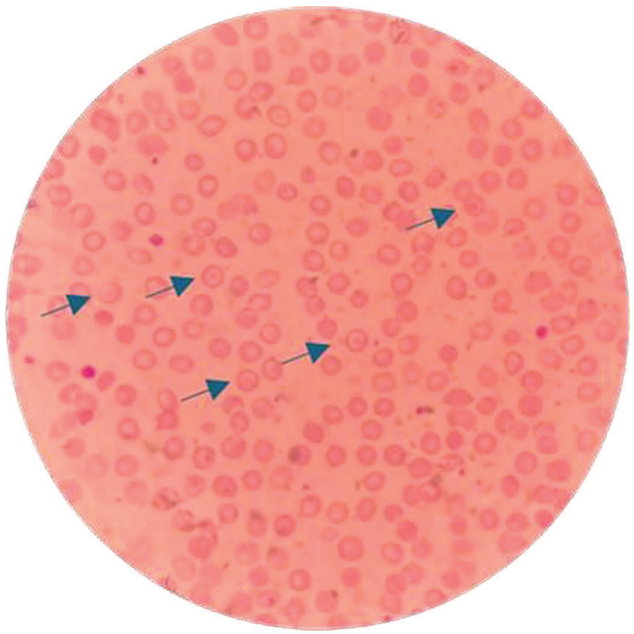

Figure 4. Examination of a peripheral blood smear with 1000x magnification showing the presence of target cells indicated by the blue arrows.

DISCUSSION

The diagnosis of hemoglobinopathy E is based on a complete blood count (CBC), specialized hematology tests, and DNA testing. A complete blood count (CBC) is the initial test commonly performed to screen for thalassemia or other hemoglobinopathies by assessing decreased MCV or MCH values, although iron deficiency anemia can also have similar symptoms. This means that the diagnosis of hemoglobinopathy cannot be based solely on MCV or MCH levels. It requires additional values, such as the MCV/RBC ratio or the Mentzer index, a peripheral blood smear, and other specialized tests such as HPLC (HbA1c) and electrophoresis, or, most effectively, DNA mutation analysis.[7]

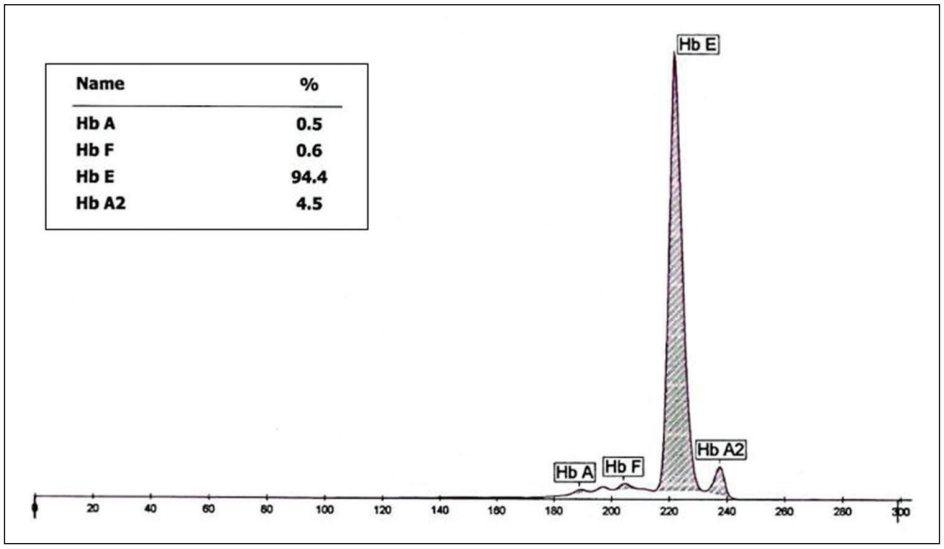

Figure 5. Electrophoresis examination showing high HbE values.

The HbA1c is a routine test for monitoring long-term blood sugar levels in patients with diabetes mellitus because it directly correlates with the risk of complications from the condition. Because it relates to hemoglobin, the HbA1c test can be influenced by factors such as erythrocyte survival time and the levels of certain hemoglobin fractions.[1,8] Because the HbA1c test is based on normal hemoglobin, the presence of other conditions such as hemoglobinopathies can affect the test results.[9] This is evident in the patient's HbA1c test results, which are still undetectable on the device even after repeating the test with dilution.

The undetectable HbA1c levels on the HPLC method used in this patient may be due to changes in the glycation process from HbA to A1c, resulting in abnormal peaks that make the A1c estimate unreliable. Hemoglobinopathy itself can make red blood cell production more susceptible to hemolysis, shortening glycosylation time, resulting in falsely low or even undetectable A1c results.[9] This is indicated by the appearance of transverse lines on two HbA1c test graphs, indicating this change (Figures 1 and 2).

Hemoglobinopathies are divided into two groups: thalassemia and hemoglobin variants.[1,8] In this patient, a high variant-window area of 93.7% was observed, with a decrease in the HbA fraction and an increase in the HbF, HbE, and HbA2 fractions, suggesting beta thalassemia with hemoglobinopathy E on the second test, after sample dilution. HPLC is an examination that separates various types of hemoglobin on a cation-exchange column and usually requires only a single sample injection. This examination is usually used as an alternative to electrophoresis examination but has the ability to identify and quantitatively measure low levels of HbA2 and HbF, although co-migration between HbA2 and HbE can occur, so that inaccurate HbA2 levels can occur due to the presence of other hemoglobin variants such as HbE, Lepore, DPunjab and S.[2,10]

The results of the HbA1c examination were then confirmed by the results of other laboratory tests, such as the patient's complete blood count, which showed a hemoglobin level of 12.1 g/dL, hematocrit 36.9%, erythrocytes 6,270,000/uL, MCV 58.9 fL, MCH 19.3 pg, leukocytes 13,370/uL, platelets 399,000/uL, and an increase in the RDW-CV value of 17.7%, indicating the possibility of variations in the shape of erythrocytes in the measured sample. Based on these results, a Mentzer index of less than 13 (9.4) indicated the possibility of the patient suffering from thalassemia. This is also supported by the peripheral blood smear, which showed hypochromic microcytic erythrocytes with target cells (+), suspicious for thalassemia. This result was further confirmed by electrophoresis, which showed a decrease in the HbA fraction, an increase in the HbF, HbE and HbA2 fractions, suggesting a beta thalassemia with hemoglobinopathy E. The uniqueness of this case is that the patient was asymptomatic, without a known family history, no signs of pancytopenia and no signs of hemolytic events or other complications, although this condition could possibly be caused by a variant form of the homozygous or heterozygous HbE E trait, which often causes clinicians to miss the attention of their diabetic patients with inappropriate HbA1c results.[11-13] The HbE condition is a thalassemic hemoglobinopathy with an abnormal HbE structure due to spontaneous point mutations in the beta-globin chain, causing substitution of lysine for glutamic acid, and the disease pattern is similar to that of beta thalassemia.[14,15]

In areas with high prevalence of Hb variants, clinical laboratory examinations need to consider various limitations of the examination, including those for HbA1c determination. The presence of other clinical conditions, such as hemoglobinopathies, can lead to silent conditions that make HbA1c levels determined by HPLC fail to reflect actual blood glucose levels, making treatment for diabetes patients becomes less appropriate.[15-17] On the other hand, HbA1c examination with HPLC is preferred

over other methods, such as immunoturbidity or point-of-care tests, because it can detect Hb variants, both homozygous and heterozygous, which can make HbA1c results less accurate.[17] Therefore, it is important to educate clinicians to avoid ordering HbA1c examinations in these patients. It is also worth noting that in regions within the thalassemia belt, such as Indonesia, hemoglobinopathy may remain undiagnosed well into adulthood in asymptomatic individuals.[6] In many individuals with HbE trait or beta-thalassemia minor, clinical manifestations may be minimal and therefore remain undiagnosed unless routine laboratory testing is performed. In this case, the hemoglobinopathy was incidentally detected during preoperative laboratory evaluation. On the other hand, the subtle microcytic picture of HbE is frequently attributed to iron deficiency, and neither electrophoresis nor HPLC is performed routinely in primary care, in the absence of clinical suspicion or family history, underscoring the need for heightened clinical awareness. Patients with Hb variants who have diabetes mellitus should be advised to use other methods of glucose monitoring that are not based on hemoglobin, such as fructosamine, glycated albumin or periodic glucose examinations to determine long-term glycemic control.[5,18-20]

CONCLUSION

Undetectable HbA1c values and the appearance of window variants on HPLC can be caused by hemoglobinopathies. This condition can be an initial indicator of Hb variants in patients with hemoglobinopathies or thalassemia traits who are clinically asymptomatic. Therefore, HbA1c testing cannot be used to monitor blood glucose levels in patients with hemoglobinopathies.

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Ethical Consideration
Patient's consent was obtained before submission of the manuscript.

Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
DPS: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Visualization, Supervision, Project administration, Funding acquisition; **SZ:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – review and editing, Visualization, Project administration, Funding acquisition; **MRF:** Methodology, Software, Validation, Formal analysis, Data curation, Writing – review and editing, Project administration, Funding acquisition.

Data Availability Statement
All data generated or analyzed during this study are included in this published article.

Author Disclosure
The authors declared no conflict of interest.

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