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NAVIGATING GLYCEMIC CONTROL IN HEMOGLOBIN VARIANTS: A CASE OF UNDETECTABLE HbA1c IN A PATIENT WITH HbE TRAIT

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ABSTRACT
Hemoglobin A1c (HbA1c) is a key marker for monitoring long-term glycemic control in diabetic patients, providing an average of blood glucose levels over 2-3 months. However, hemoglobinopathies, such as hemoglobin E (HbE), can interfere with the accuracy of HbA1c measurements, leading to diagnostic confusion. This case study discusses a 21-year-old female with an undetectable HbA1c result, despite normal glucose levels and no history of diabetes. Further investigation using hemoglobin electrophoresis revealed the presence of the HbE variant, confirming a heterozygous HbE trait. While the patient was asymptomatic, the discovery of HbE emphasized the need for careful interpretation of HbA1c in patients with hemoglobin variants. This case highlights the diagnostic challenges posed by HbE in diabetes management and the importance of alternative glycemic assessment methods, such as fructosamine or continuous glucose monitoring. Additionally, identifying the HbE trait is crucial for managing potential genetic risks, especially in regions with a high prevalence of hemoglobinopathies, and underscores the importance of genetic counseling for reproductive planning.

KEYWORDS: Glycated hemoglobin, HbE heterozygous, Bio-Rad D10, Hb Electrophoresis.

INTRODUCTION
HbA1c is a key marker used to monitor long-term blood glucose levels in diabetic patients, as it measures the percentage of glycated hemoglobin in the blood, reflecting average glucose concentrations over the previous 2-3 months.[1] This makes it an essential tool for assessing diabetes management, providing a more stable indicator compared to daily glucose tests, which can vary throughout the day.[2] However, certain hemoglobinopathies—genetic variations in hemoglobin—can interfere with the accuracy of HbA1c measurements by altering the structure or lifespan of red blood cells, leading to either falsely elevated or undetectable HbA1c levels. These hemoglobin variants, such as sickle cell hemoglobin (HbS) and hemoglobin E (HbE), result from mutations in the genes coding for the globin chains (alpha or beta) and can cause mild to severe blood disorders, complicating diagnosis and treatment.[3] Some variants may cause anemia, alter oxygen transport, or interact with other hemoglobinopathies, which underscores the importance of identifying them to avoid diagnostic confusion and mismanagement.

The HbE trait results from a single-point mutation in the β-globin gene, where glutamic acid is replaced by lysine at position 26 of the β-globin chain. This mutation leads to the production of hemoglobin E, a structurally altered form of hemoglobin that behaves slightly differently from normal hemoglobin (HbA).[4] While individuals with the HbE trait (heterozygous HbE) are usually asymptomatic, they may exhibit mild anemia or microcytosis, characterized by smaller, paler red blood cells.[5] This often goes unnoticed unless discovered through routine blood tests or hemoglobin electrophoresis. HbE, on its own, does not typically cause serious health concerns, but it becomes clinically significant when combined with other genetic hemoglobin disorders. In particular, when individuals with the HbE trait inherit a β-thalassemia gene (HbE/β-

thalassemia), the result can be a more severe form of anemia that may require ongoing medical management, including regular blood transfusions, iron chelation therapy, and possibly bone marrow transplantation in severe cases.[6] HbE/β-thalassemia is considered one of the most common forms of thalassemia in Southeast Asia and can range from mild to severe in its clinical presentation. Awareness and identification of the HbE trait through genetic screening are particularly important in populations with a high prevalence of hemoglobinopathies to guide reproductive decisions and manage potential risks in offspring.[7,8] Additionally, individuals with the HbE trait may show altered results in HbA1c measurements used for diabetes monitoring, as the presence of abnormal hemoglobin can interfere with the accuracy of these tests, further highlighting the importance of recognizing and accounting for HbE in clinical practice.

The Bio-Rad D-10 is an automated analyzer that uses HPLC methodology to accurately measure HbA1c, a key marker for managing diabetes. It offers fast, precise results with minimal sample preparation, making it ideal for routine testing in clinical labs. In addition to HbA1c, the D-10 can also detect certain hemoglobin variants, ensuring reliable results even in patients with abnormal hemoglobin types. Its efficiency and versatility make it a valuable tool for both high- and low-throughput laboratories.[9]

CASE

A 21-year-old female presented to the outpatient department for routine laboratory testing as part of a general health check-up. She had no significant symptoms or known medical history. Her physical examination was unremarkable, and she was in good health. The initial laboratory results, including complete blood count (CBC), fasting blood sugar (FBS), post prandial blood sugar (PPBS), urea, creatinine and thyroid function tests, were all within normal ranges. However, the HbA1c level was found to be undetectable by instrument on re-running the sample still HbA1c was undetectable, despite the patient having no signs or history of diabetes or related metabolic conditions. Given this discrepancy, her physician ordered further investigations to determine the cause of the abnormal HbA1c level. Hemoglobin electrophoresis was performed, which revealed the presence of the HbE variant. The patient was found to be heterozygous for the HbE variant, a condition not previously diagnosed.

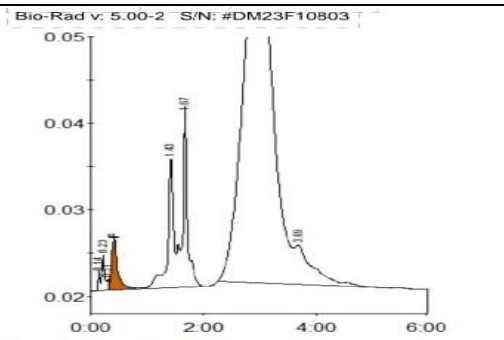
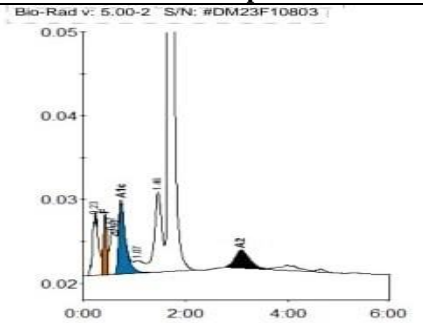
Diagnosis

In this case, the patient's diagnosis was confirmed as heterozygous for the HbE variant after an undetectable HbA1c result led to further testing. Despite having normal fasting and postprandial blood glucose levels, Liver function test, Renal function test, Thyroid hormone, Hemoglobin, Bilirubin, Total Red blood cell, and Platelet count **shown in table 1**, and no prior history of diabetes or metabolic disorders. **Table 2 shows** A1c value detected in D10 for control patient and for HbE trait patient A1c hasn't been detected this clearly confirms that Hb variant affects HbA1c values and the retention time (RT) for A1c has been shown in **table 3**. HbA1c relies on the glycation of normal hemoglobin (HbA), but in individuals with hemoglobin variants, the altered structure or quantity of normal hemoglobin leads to unreliable readings. **Figure** 1, clearly shows that hemoglobin electrophoresis A2 peak results 27.8% (reference range 25-35% for trait) and F peak shows <0.8% (reference range <1%) this confirmed the presence of the HbE heterozygous for this condition. While the patient does not present with symptoms typically associated with HbE or other hemoglobinopathies, this case highlights the need for careful interpretation of HbA1c levels in such patients, as standard diabetes monitoring tools may not be accurate.

Table 1: Results of basic biochemistry, haematology, and hormone profile on routine health checkup.

Parameters	Results	Reference range	Methodology
FBS	89	70 - 110 mg/dl	Hexokinase
PPBS	135	110 - 140 mg/d	Hexokinase
Urea	36	17 – 43 mg/dl	GLDH, Kinetic Assay
Creatinine	0.99	0.81 - 1.44 mg/dL	Jaffe-Kinetic
SGOT	31	0 - 50 U/L	IFCC
SGPT	18	0 - 50 U/L	IFCC
ALP	111	30 - 120 U/L	IFCC
Total Protein	6.7	6.6 - 8.7 gms/dl	Biuret
Total Bilirubin	1.03	0.2 - 1.2 mg/dL	Diazo
Direct Bilirubin	0.34	0.0 - 0.4 mg/dl	Diazo
Haemoglobin	13.15	12 - 16 gms%	Photometric measurement
Total RBC count	4.34	3.8 - 4.8 millions/mm3	Coulter principle
Platelet count	226.6	150 - 450 10^3/µl	Coulter principle
Free T3	3.82	2.5 - 3.9 pg/mL	CLIA
Free T4	0.810	0.6 - 1.1 ng/dL	CLIA
TSH	1.046	0.34 - 5.2 µIU/mL	CLIA

Table 2: Shows that HbE patient doesn't have the A1c value, whereas normal patient shows the A1c value.

HbE Trait Patient	Control patient
	

Peak table - ID: TILOOMA_ROY

Peak	R.time	Height	Area	Area %
Unknown	0.14	2295	4986	0.3
A1a	0.23	4156	14521	0.8
A1b	0.31	1351	4082	0.2
F	0.41	6020	42230	2.3
P3	1.43	15002	106609	5.7
A0	1.67	21016	128033	6.9
Unknown	2.98	39926	1457366	78.4
Unknown	3.69	4587	101614	5.5
Total Area:		1859439		

Concentration:	%
F	2.3

Peak table - ID: A2CTRL

Peak	R.time	Height	Area	Area %
A1a	0.23	7654	58313	3.1
F	0.43	7208	34567	1.9
Unknown	0.57	5068	31333	1.7
LA1c/CHb-1	0.63	4700	11578	0.6
A1c	0.75	8389	80472	5.9
Unknown	1.07	1522	20215	1.1
P3	1.46	9541	96024	5.2
A0	1.67	286686	1475649	79.7
A2	3.09	2138	43595	2.4
Total Area:		1851746		

Concentration:	%
F	1.9
A1c	5.9
A2	2.4

Table 3: shows the retention time (RT) for each parameter and HbA1c peak has to be come between 0.66 – 1.00 min.

Peak Name	RT (min.)	Window (min.)
A1a	0.21	0.16-0.26
A1b	0.29	0.23-0.35
F	0.485	0.41-0.56
LA1c/CHb-1	0.715	0.56 - 0.87
LA1c/CHb-2	0.735	0.60 - 0.87
A1c	0.83	0.66 - 1.00
P3	1.43	1.23 - 1.63
A0	1.70	1.55 - 1.85
A2	3.01	2.59 - 3.43
S	4.16	4.02 - 4.30
C	4.80	4.70 - 4.90

Complications

While individuals with the HbE heterozygous trait typically remain asymptomatic and do not require treatment, complications can arise when HbE is inherited alongside another hemoglobinopathy, such as β-thalassemia. The combination of one HbE gene and one β-thalassemia gene results in HbE/β-thalassemia, a condition that can range from mild anemia to severe thalassemia.[10,6] Potential complications of HbE/β-thalassemia include moderate to severe anemia due to ineffective red blood cell production and hemolysis, jaundice from the increased breakdown of abnormal red blood cells, growth retardation in children due to chronic anemia, and splenomegaly from excessive red blood cell destruction.[11] Patients with HbE/β-thalassemia may require regular blood transfusions, iron chelation therapy, and other supportive treatments based on the severity of their symptoms.

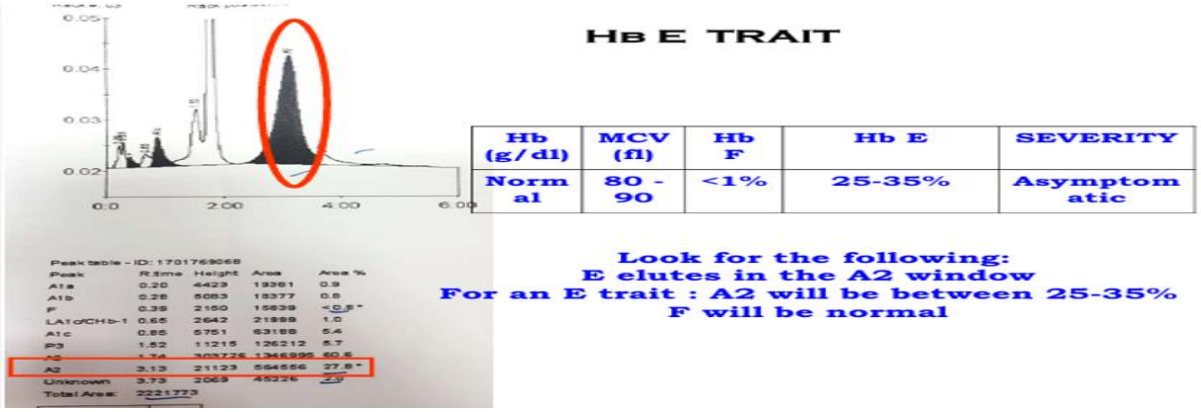


Figure 1: shows the level of A2 27.8% and F <0.8%. Confirms the presence of HbE variant.

DISCUSSION

This case underscores the impact of hemoglobin variants, specifically HbE, on the accuracy of HbA1c measurements, a key tool for long-term diabetes management. The patient's undetectable HbA1c, despite having normal blood glucose levels and no symptoms of diabetes, was identified as a result of the HbE variant. Hemoglobin E alters the structure of hemoglobin and can interfere with the glycated hemoglobin assay, leading to falsely low or undetectable HbA1c levels. This situation demonstrates the importance of recognizing and accounting for hemoglobinopathies when interpreting HbA1c results.

In patients with hemoglobin variants like HbE, traditional HbA1c testing may not reliably reflect glycemic control, necessitating alternative testing methods such as fructosamine test in continuous glucose monitoring for accurate assessment.[12,13], fructosamine results are based on the glycation of serum proteins and are unaffected by hemoglobin variants.[14] While HbE itself is usually asymptomatic and benign, it becomes clinically significant when co-inherited with other hemoglobinopathies, such as β-thalassemia, which can result in more severe conditions like HbE/β-thalassemia. The HbE variant affects HbA1c levels through several mechanisms. First, it alters the beta-globin chain (Glu26Lys mutation), potentially reducing glycation sites, which leads to lower HbA1c formation. Additionally, HbE is associated with mild hemolysis, shortening the lifespan of red blood cells (RBCs). Since HbA1c reflects average blood glucose over the RBC lifespan, a reduced lifespan results in lower HbA1c levels, even when blood glucose levels are normal or elevated. The HbE variant may also affect the glycation kinetics, slowing the rate of glycation and further reducing HbA1c formation. Furthermore, HbE can interfere with certain HbA1c measurement techniques, particularly those based on charge differences, leading to inaccurate readings.[15, 16]

Furthermore, identifying the HbE variant through hemoglobin electrophoresis in this case was crucial for proper diagnosis and management. It also highlights the importance of genetic counselling for individuals with HbE, particularly in regions with a high prevalence of hemoglobin disorders. Genetic counselling can assist in understanding the implications of HbE and guide family planning to mitigate risks of severe hemoglobinopathies in future generations. This case reinforces the need for a comprehensive approach to diabetes management and genetic risk assessment in patients with abnormal hemoglobin types.

CONCLUSION

This case underscores the diagnostic challenges posed by hemoglobin variants, such as HbE, in routine HbA1c testing. The patient's undetectable HbA1c level, despite normal glycemic markers, was attributed to the HbE trait, a hemoglobinopathy that affects the accuracy of glycated hemoglobin measurements. The discovery of the HbE variant through hemoglobin electrophoresis highlighted the need for alternative testing methods for accurate glycemic control in such patients. Additionally, while individuals with heterozygous HbE are typically asymptomatic, identifying the trait is essential for managing potential genetic risks, especially in populations with a high prevalence of hemoglobin disorders. Genetic counselling should be considered in these cases to guide reproductive decisions and prevent complications in future generations.

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This study did not receive any funding.

Conflict of Interest

All authors declare no conflict of interest.

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