

THE IMPACT OF HEMOGLOBIN J (HbJ) VARIANT ON HbA1c MEASUREMENT: A CASE REPORT

Hemoglobin J (HbJ) Varyantının HbA1c Ölçümü Üzerindeki Etkisi: Bir Olgu Sunumu

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ABSTRACT

In this case report, discrepancies in HbA1c measurements were observed in a 54-year-old female patient with Type 2 diabetes due to the presence of a haemoglobin (Hb) variant, most likely HbJ. HbA1c were measured as 10.5% using capillary electrophoresis(CE), 8.92% using ion-exchange high-performance liquid chromatography(HPLC), and 10.4% using immunoassay. The lower HbA1c value obtained with the HPLC method suggested interference from the Hb variant(most likely HbJ detected in the Z13 region). Hb variants can lead to inaccurate HbA1c results, which may influence clinical decisions, particularly in diabetes management. This case highlights that CE can be a reliable method even in the presence of Hb variants, but results from different methods should be interpreted with caution. Due to the poor glycemic control, the treatment plan was revised, and the patient was recommended for monitor the clinical implications of the Hb variant. In conclusion, considering Hb variants in the interpretation of HbA1c is critical to prevent misdiagnosis and inappropriate treatment.

Keywords: Hb J; High-Performance Liquid Chromatography; HbA1c; Capillary Electrophoresis; Immunoassay

ÖZET

Bu olgu sunumunda, Tip 2 diyabetli 54 yaşındaki kadın hastada hemoglobin (Hb) varyantı (muhtemel HbJ) nedeniyle HbA1c ölçümlerinde farklılıklar gözlemlenmiştir. HbA1c düzeyi kapiller elektroforez (CE) yöntemiyle %10,5, iyon değişimli yüksek performans sıvı kromatografisi(HPLC) yöntemiyle %8,92 ve immünoassay yöntemiyle %10,4 olarak ölçülmüştür. İyon değişimli HPLC yöntemiyle elde edilen daha düşük HbA1c değeri, Hb varyantının (Z13 bölgesinde tespit edilen muhtemel HbJ) interferans etkisini düşündürmektedir. Hb varyantları, özellikle diyabet yönetiminde klinik kararları etkileyebilecek hatalı HbA1c sonuçlarına yol açabilir. Bu olgu, Hb varyantlarının varlığında bile kapiller elektroforezin güvenilir bir yöntem olabileceğini, ancak farklı yöntemlerle elde edilen sonuçların dikkatle değerlendirilmesi gerektiğini göstermektedir. Hastanın glisemik kontrolünün yetersiz olması nedeniyle tedavi planı yeniden düzenlenmiş ve Hb varyantının klinik etkilerinin izlenmesi amacıyla takibe alınması önerilmiştir. Sonuç olarak, HbA1c sonuçlarının yorumlanmasında Hb varyantlarının dikkate alınması, yanlış tanı ve uygunsuz tedavilerin önlenmesi açısından büyük önem taşımaktadır.

Anahtar Kelimeler: Hb J; Yüksek Performanslı Sıvı Kromatografisi; HbA1c; Kapiller Elektroforez; İmmünoassay

INTRODUCTION

In cases of clinical discrepancy, it is appropriate to repeat the analysis using a sample collected and transferred under standard conditions (i.e., cold chain). Glycated hemoglobin (HbA1c), formed through non-enzymatic glycation of hemoglobin (Hb) molecules, quantitatively reflects mean plasma glucose levels over the erythrocyte lifespan (approximately 120 days), with the strongest correlation to the preceding 2-3 months, establishing its role as the gold standard for long-term diabetes management assessment. HbA1c is considered the gold standard for the diagnosis and evaluation of diabetes. Hemoglobinopathies are prevalent in numerous regions around the world. Over 900 variants of Hb have been identified, accounting for at least 43% of all possible single-point mutations. HbJ is a less common alpha-globin gene variant that can affect HbA1c results (1, 2).

Clinical laboratories can determine HbA1c levels using various principal methodologies, including enzymatic analysis, immunoturbidimetric analysis, boronate affinity chromatography, capillary electrophoresis (CE), and ion-exchange high-performance liquid chromatography (HPLC). Ion-exchange HPLC and CE are among the commonly used methods for HbA1c measurement. HbA1c measurement relies on

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methodologies that exploit differences in the affinity, charge, and immune response of glycosylated Hb molecules (3, 4). Both anion- and cation-exchange HPLC and CE methods can detect the presence of Hb variants. Hemoglobin J was first characterized in 1963 by Baglioni and Weatherall, who identified the variant in an African American family (5). Although HbJ carriers generally show no hematologic abnormalities, this variant causes significant analytical interference in HPLC-based HbA1c testing, potentially yielding unreliable glycemic monitoring results. Several studies have detailed the effects of silent hemoglobinopathy variants on HbA1c values; variants such as Hb Camden, Hb Austin, and Hb N-Baltimore have been reported to cause falsely elevated HbA1c results (6). If an HbA1c result does not correlate with a patient's clinical condition, hemoglobinopathies and conditions affecting red blood cell lifespan should be considered as potential causes.

In this case report, we characterize a suspected HbJ case detected via CE with an accompanying electropherogram and describe the implications of this Hb variant on the accurate interpretation of HbA1c results obtained using HPLC and immunoassay methods.

CASE REPORT

A 54-year-old female patient with a diagnosis of Type 2 diabetes was undergoing routine follow-up. During her routine check-up, her fasting blood glucose was found to be 192 mg/dL, Hb level was 14.1 g/dL, and TSH level was within normal limits at 1.33 µIU/mL. HbA1c was measured as 10.5% using the CE method (Sebia Capillarys 3, France), and an atypical Hb variant profile was observed on the CE electropherogram (Figure 1). To confirm the Hb variant and conduct a detailed analysis, repeat CE Hb variant analysis was performed (Figure 2). Further investigation revealed a variant in the Z13 region, suggesting a rare Hb variant, most likely HbJ. Genetic analysis to determine the specific HbJ variant type could not be performed. The patient's medications included Ryzodeg (insulin degludec + aspart), Galvus Met (vildagliptin + metformin), and Jardiance (empagliflozin). Written informed consent was obtained from the patient for publication of this case report.

The same blood sample was reanalysed for HbA1c using two different methods: ion-exchange HPLC (Lipotronic HPLC System GH900, China) and immunoassay (Cobas 8000, Roche Diagnostics, Germany). HbA1c values were 8.92% and 10.4%, respectively.

Laboratory Findings:

- HbA1c: 10.5% (92 mmol/mol) (capillary electrophoresis)
- HbA1c: 8.92% (74 mmol/mol) (ion-exchange HPLC)
- HbA1c: 10.4% (92 mmol/mol) (immunoassay)
- Fasting Blood Glucose: 192 mg/dL (10.71 mmol/L)
- Hb: 14.1 g/dL (previous result: 14.0 g/dL)
- TSH: 1.33 µIU/mL
- Hb Variant Analysis: Variant in Z13 region, most likely HbJ (Figure 2).

DISCUSSION

HbA1c is formed by the reaction between glucose and the NH₂-terminal valine of the Hb beta chain. It has been used as the gold standard in the clinical management of diabetes for approximately 30 years for routine monitoring of glycemic status. Hb variants can cause falsely elevated or decreased HbA1c results in some measurement methods. However, the CE method used in this case is considered reliable in the presence of hemoglobinopathies. The patient's high fasting blood glucose level was consistent with the HbA1c value, suggesting that the measurement was valid despite the variant.

The National Glycohemoglobin Standardization Program (NGSP) considers HPLC the gold standard for HbA1c analysis, while the International Federation of Clinical Chemistry (IFCC) recommends CE (7, 8). Both CE and ion-exchange HPLC can incidentally detect Hb variants, which may interfere with HbA1c assays. Immunoassays, boronate affinity chromatography, and enzymatic methods are generally less likely to detect such variants. In this case, we measured HbA1c using ion-exchange HPLC, immunoassay, and CE. The results suggested that the HbJ variant negatively interfered with the HPLC method. Barakat et al. also reported a case where ion-exchange HPLC yielded a negatively biased result due to a variant (9). Clinical biochemists must recognize factors that may lead to spuriously high or low HbA1c measurements and should collaborate with clinical departments when an abnormal Hb

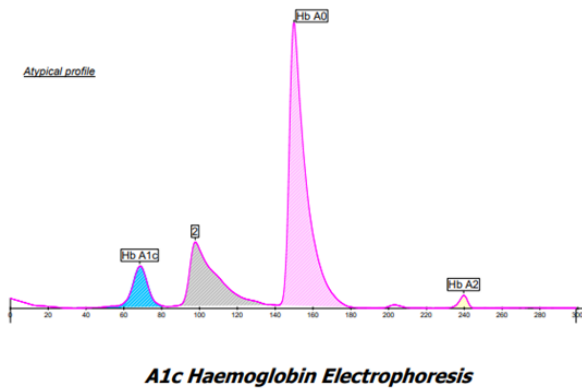


Figure 1. Capillary electrophoresis performed for HbA1c measurement revealed an atypical hemoglobin variant detection in the electrophoretogram. An unknown peak was observed in region number two.

variant is suspected.

The patient’s clinical course indicated poorly controlled Type 2 diabetes despite current treatment. The high HbA1c value and fasting blood glucose level of 192 mg/dL suggested inadequate glycemic control. HbA1c levels were 10.5% by CE, 8.92% by HPLC, and 10.4% by immunoassay, all indicating poor diabetes control. In this case, there was no significant clinical discrepancy between glucose levels and HbA1c values across methods, although the HPLC result was lower. However, such consistency may not occur in all cases, potentially leading to errors in diabetes management based on HbA1c. Therefore, both clinical biochemists and clinicians should exercise caution when encountering discrepancies between HbA1c and glucose levels or when different methods yield inconsistent results.

HbJ has various characteristics and functions, with variable effects on HbA1c levels. The most common HbJ variant, HbJ Capetown ($\alpha 2$ 92Gln $\beta 2$), causes falsely elevated HbA1c levels, as does HbJ Valencia, while HbJ Baltimore and HbJ Meerut cause falsely low HbA1c values. No Reports indicate that the HbJ variant can lead to either spuriously high or low HbA1c results, depending on the analytical method used (9- 11). In a recent study, Song et al. investigated the interference of Hb Q-Thailand, Hb G-Honolulu, Hb Ube-2, Hb New York, Hb J-Bangkok, Hb G-Coushatta, and Hb E variants

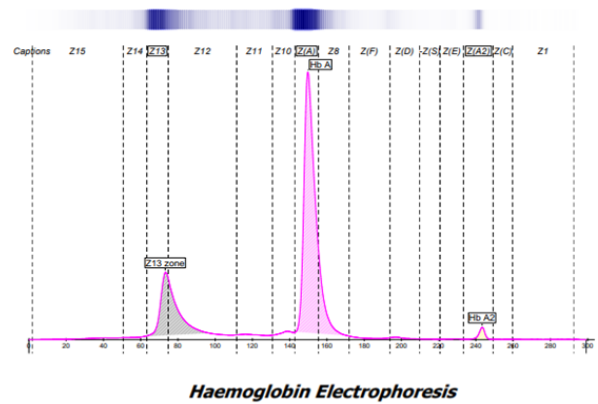


Figure 2. A high probability HbJ variant was detected in the Z13 region of the capillary electrophoresis for hemoglobin variant analysis.

on HbA1c measurement using five different methods (Intelligene Biosystems QuanTOF, Sebia Capillarys 3 TERA, Premier Hb9210, Arkray HA-8190V, Tosoh G11, and Bio-Rad D-100). In the evaluation of Hb J-Bangkok, LC-MS/MS served as the reference method. Among six comparative methods, interference was observed in five—including CE, boronate affinity chromatography, and three HPLC techniques—highlighting method-dependent variability in HbA1c measurement. (12).

A limitation of our case was the inability to confirm the suspected HbJ variant (Z13 zone variants) through genetic testing. Variants in the Z13 zone detected by CE include Hb AJ-Ain Abu Dhabi, HbJ-Europa, HbJ-Baltimore, HbJ-Ravigo, HbJ-Lome, HbJ-Norfolk, HbJ-Nigeria, and HbJ-Kaohsiung.

As a result, this case highlights the utility of CE for HbA1c measurement in the presence of Hb variants (HbJ and Z13 zone) and underscores the importance of treatment optimization in poorly controlled diabetes management. The suspected HbJ variant in the Z13 region is a rare mutation, and its clinical significance should be evaluated through patient follow-up.

CONCLUSION

Rigorous interpretation of HbA1c results is crucial to avoid misleading clinical conclusions, particularly in certain populations with a relatively high prevalence

of Hb variants. Additionally, it necessitates method-specific validation of HbA1c measurements to ensure accuracy. Routine laboratory tests can be affected by various Hb variants, which is an important consideration. HbA1c results that do not correlate clinically or with blood glucose levels should raise suspicion of a Hb variant and should be confirmed using different methods. In patients with Hb variants, an appropriate HbA1c measurement method should be selected and consistently used for follow-up. This is critical to prevent misdiagnosis and mismanagement of patients. Interferences in HbA1c measurement can lead to incorrect diagnosis and treatment.

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