

## CASE REPORT

# Detection of a Silent Hemoglobin Variant During HbA1c Monitoring: the Crucial Role of Capillary Electrophoresis

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### ABSTRACT

**Background:** Hemoglobin A1c (HbA1c) is a widely accepted marker for long-term glycemic control. However, the presence of hemoglobin variants can interfere with its measurement, depending on the analytical method used. Accidental detection of silent variants during HbA1c monitoring is a known challenge in clinical laboratories.

**Methods:** A 79-year-old male patient with no known history of diabetes or hemoglobinopathy was admitted for chest pain. Routine laboratory tests were performed. HbA1c measurement was initially requested and performed using capillary electrophoresis (Capillarys OCTA 3, Sebia) in HbA1c mode. Confirmatory investigations included hemoglobin electrophoresis in both alkaline and acidic media, as well as High-Performance Liquid Chromatography (HPLC) using the ADAMS A1c HA-8180V (Arkray).

**Results:** Routine biochemical and hematological parameters were within normal ranges. The initial capillary electrophoresis analysis yielded an atypical electrophoretic profile characterized by split peaks for both HbA0 and HbA1c fractions. A minor abnormal fraction (2.3%) was also detected. Due to this interference, no HbA1c value could be reported. In contrast, further analysis using HPLC and standard gel electrophoresis returned normal results, suggesting the presence of a variant detectable specifically under the separation conditions of the capillary method used.

**Conclusions:** This case illustrates the potential for analytical interference by a rare hemoglobin variant, which altered the electrophoretic migration of HbA0 and HbA1c in capillary electrophoresis. The discrepancy between techniques highlights the importance of using complementary methods to ensure diagnostic accuracy when an atypical profile is observed. In such situations, alternative markers like fructosamine or glycated albumin should be considered for glycemic monitoring.

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### KEYWORDS

HbA1c, hemoglobinopathy, capillary electrophoresis, diabetes

### INTRODUCTION

Hemoglobin is a metalloprotein essential for oxygen transport in the human body and represents the main component of red blood cells. Among adults, the predominant form is hemoglobin A (HbA), a tetrameric molecule composed of two  $\alpha$  and two  $\beta$  chains ( $\alpha_2\beta_2$ ). The genes responsible for the synthesis of these chains

are located on two distinct loci: the  $\alpha$ -globin gene cluster on chromosome 16 and the  $\beta$ -globin gene cluster on chromosome 11 [1].

Among the various physiological functions of hemoglobin, its non-enzymatic ability to bind glucose is leveraged to assess long-term glycemic control. This glycation process leads to the formation of HbA1c, now widely recognized as a reference biomarker for monitoring diabetes mellitus [2].

Although HbA1c measurement has been internationally standardized (IFCC, NGSP), it remains susceptible to several types of interference, especially in the presence of hemoglobin variants that may alter results depending on the analytical method employed [3].

These interferences, often clinically silent, may modify the separation profile of hemoglobin fractions, thereby compromising result interpretation - particularly with certain techniques such as capillary electrophoresis [4]. We present here a clinical case highlighting the incidental discovery of an atypical electrophoretic profile during an HbA1c assay, revealing the potential impact of hemoglobin variants on biochemical analysis and underscoring the importance of critical interpretation of results.

## CASE PRESENTATION

A 79-year-old man with no known medical history of diabetes, hemoglobinopathy, or hematological disorders, presented to the emergency department with chest pain. As part of the diagnostic workup, routine laboratory tests were performed, including a complete blood count (CBC), serum electrolyte panel, liver and kidney function tests, and HbA1c measurement.

The CBC showed a hemoglobin level of 14.4 g/dL, with no microcytosis (Mean Corpuscular Volume [MCV] = 93 fL) or hypochromia (Mean Corpuscular Hemoglobin Concentration [MCHC] = 34.3 g/dL). Fasting plasma glucose was 0.90 g/L (5.0 mmol/L). All other laboratory parameters were within normal limits.

HbA1c testing was performed using the Capillarys Octa 3 (Sebia) system (HbA1c mode). The result showed the following fractions: HbA0 = 90.3%, HbA2 = 2.1%, and an "Other Hb" fraction at 2.3%. No HbA1c value was reported, as the system flagged the profile as atypical. Electropherogram analysis revealed a split peak in both HbA0 and HbA1c, suggesting the presence of an abnormal hemoglobin variant (Figure 1). Notably, previous HbA1c analyses performed using HPLC ADAMS A1c HA-8180V (Arkray) had never shown chromatographic abnormalities and were consistently interpreted as normal. Further investigations were conducted in our laboratory. Hemoglobin electrophoresis was performed in alkaline medium using Capillarys Octa 3 (Sebia) (hemoglobin mode), and in acidic medium using Hydrasis 2 Scan Focusing (Sebia) (Figure 2A); both results were normal. HbA1c measurement by HPLC ADAMS A1c HA-8180V (Arkray) was also normal (Figure 2B).

## DISCUSSION

The measurement of HbA1c relies on various analytical approaches, which can be broadly classified into two categories: non-separation methods and separation methods. Non-separation techniques, such as immunoassays, target a specific portion of the  $\beta$ -globin chain and are therefore unable to detect abnormal hemoglobins. Consequently, in the presence of structural variants, they may provide falsely normal results.

In contrast, separation techniques - such as HPLC and capillary electrophoresis - offer superior discrimination of hemoglobin profiles. HPLC separates hemoglobin fractions based on their ionic charge and detects them spectrophotometrically. While it is sensitive to common variants, its ability to differentiate may be limited by co-elution phenomena, particularly with the HbA0 fraction [5].

Capillary electrophoresis, on the other hand, separates hemoglobin based on its electrophoretic mobility in an alkaline buffer. The HbA1c mode of the Capillarys Octa 3 system (Sebia) enables simultaneous detection of glycosylated and non-glycosylated forms and can identify migratory variants, especially those co-migrating with HbA0. HbA1c is calculated using the IFCC reference formula:  $\text{HbA1c (\%)} = \text{HbA1c} / (\text{HbA1c} + \text{HbA0}) \times 100$ , where HbA0 represents the non-glycosylated fraction [6,7].

Beyond its application in glycemic control, capillary electrophoresis in HbA1c mode can also serve as an indirect screening tool for hemoglobin variants. Abnormal migration profiles - such as atypical peaks not corresponding to HbA0, HbA1c, or HbA2 - or split peaks may indicate the presence of a hemoglobin variant.

A reliable phenotypic diagnosis of hemoglobinopathies generally requires the combination of several complementary analytical methods [8,9]. Hemoglobin electrophoresis alone is considered insufficient and must be supplemented with at least two, ideally three, distinct techniques. French recommendations emphasize the use of multiple methods to identify hemoglobin variants rigorously [9], and international guidelines advise that a diagnosis based on a single method should be considered presumptive and confirmed by a second technique using a different analytical principle [8]. This multi-method strategy minimizes misinterpretation due to co-migration or co-elution phenomena and strengthens diagnostic accuracy, with referral to expert laboratories when needed.

In our case, the hemoglobin variant was incidentally detected only during the HbA1c measurement by capillary electrophoresis in HbA1c mode. No abnormalities were found in conventional hemoglobin mode nor with HPLC. This discrepancy may be explained by differences in buffer composition. The HbA1c-mode buffer utilizes borate anions. These anions interact specifically with glucose moieties, creating a charge difference that allowed the separation of the variant from the HbA0 fraction - a separation that charge-based HPLC could not achieve [10]. Conversely, the hemoglobin mode

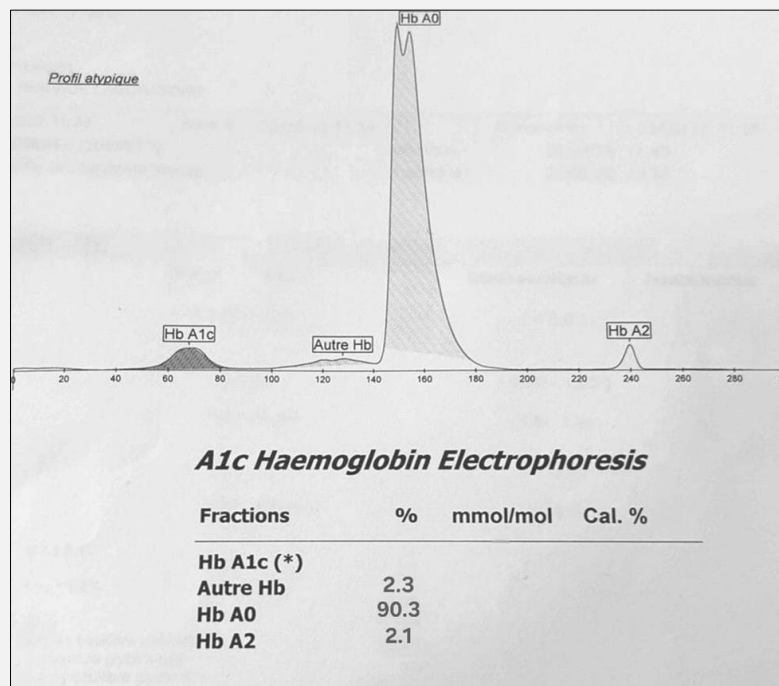


**Figure 1.** Analysis of HbA1c of this patient on Capillarys Octa 3 (Sebia) HbA1c mode.

The electropherogram reveals an atypical profile with a split peak co-migrating with HbA0 and HbA1c. Consequently, no HbA1c value was reported by the system.

buffer is optimized for genetic variant separation and does not specifically target glycated forms.

Therefore, some hemoglobin variants may exhibit different electrophoretic behavior depending on the separation medium. In our observation, the interfering variant altered the migration of both HbA0 and HbA1c, rendering HbA1c calculation impossible. Although undetectable by other techniques, its presence was real and detectable only under specific analytical conditions. An interpretive comment was included in the report to ensure clear clinico-biological communication:

“HbA1c value cannot be reported due to interference from a hemoglobin variant. It is recommended to use an alternative glycemic marker (e.g., fructosamine or glycemic profile).”

A similar case was reported in Italy in an 86-year-old diabetic patient, where a double peak involving HbA0 and HbA1c led to the identification of Hb Novara in the heterozygous state [10]. Other variants, such as Hb La Désirade and Hb Mélusine, exhibit similar behavior by co-migrating with HbA0 and escaping detection by conventional methods [11,12]. Although molecular charac-

terization was not performed in this case, the electrophoretic profile strongly resembles variants such as Hb Novara or Hb La Désirade, known for co-eluting with HbA0 on HPLC.

Such profiles highlight the limitations of isolated analytical techniques and reinforce the value of a multi-method approach combining different separation principles. This strategy increases the likelihood of detecting rare or subtle variants and prevents misinterpretation, particularly in the context of glycemic monitoring or hemoglobinopathy screening.

In patients with hemoglobinopathies interfering with HbA1c measurement, the use of an alternative glycemic marker is recommended. Fructosamine measurement is a useful marker for medium-term glycemic control. Although not standardized and less well correlated with the risk of complications than HbA1c, it remains clinically relevant in patients with hemoglobin variants. Other options, such as serum glycated albumin or continuous glucose monitoring, may also be considered in cases where HbA1c is unreliable [5].

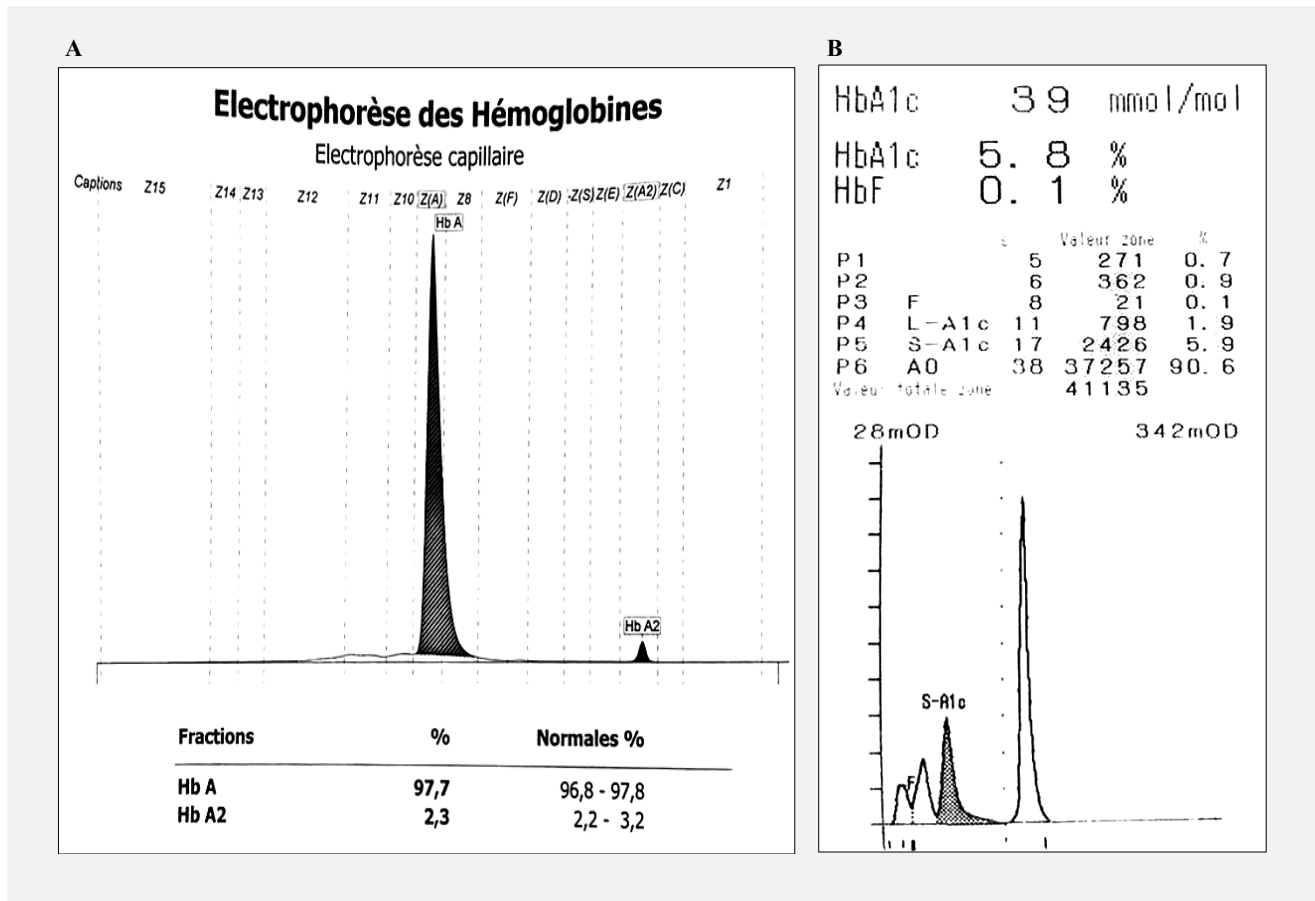


**Figure 2. Comparison of alternative analytical methods showing normal results.**

**A:** Hemoglobin electrophoresis on Capillarys Octa 3 (Sebia) in standard hemoglobin mode, displaying a normal profile (HbA 97.7%) without visible separation of the variant. **B:** HbA1c analysis of the same patient using HPLC on ADAMS A1c HA-8180V (Arkray), showing a normal chromatogram and a falsely normal HbA1c result of 5.8% (39 mmol/mol).

## CONCLUSION

The detection of hemoglobin variants represents a major challenge in clinical laboratory practice, both for diagnostic purposes and for the reliable interpretation of key biomarkers such as HbA1c. Certain atypical forms may interfere with standard analytical methods, thereby compromising the validity of reported results.

Clinicians and biologists must be aware that a “normal” HbA1c result on HPLC does not exclude the presence of hemoglobin variants. In discordant clinical situations, switching to a separation method based on a different principle, such as capillary electrophoresis, is mandatory to prevent mismanagement of diabetic patients.

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