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CASE REPORT

# Undetectable Glycated Haemoglobin Associated with Asymptomatic Sickle Cell Disease: A Case Report

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**Summary**

Glycated haemoglobin (HbA1c) testing is indicated for the screening and therapeutic monitoring of diabetes mellitus (DM). A 42-year-old woman was found to have undetectable glycated haemoglobin (HbA1c) with the Cobas C311® analyser during screening for diabetes mellitus. The fasting plasma glucose was normal. Haemoglobin (Hb) phenotyping by automated cellulose acetate electrophoresis revealed HbSC disease. The diagnosis of HbSC was confirmed by high-performance liquid chromatography (HPLC) and capillary electrophoresis. The presence of haemoglobinopathies can affect the results of HbA1c assays. In populations with a high prevalence of haemoglobinopathies, abnormally low or discordant HbA1c results should raise a suspicion of the presence of haemoglobinopathies. Alternative assay methodologies or biomarkers may be required for proper clinical management in such cases.

**Keywords: Diabetes mellitus, Glycated haemoglobin, Haemoglobinopathy, Sickle Cell Disease.**

## Introduction

Glycated haemoglobin (HbA1c) is a biomarker used for the screening, diagnosis and therapeutic monitoring of Diabetes mellitus (DM). [1] It indicates the mean plasma glucose level over two to three months preceding the analysis. [1] Glycated haemoglobin is formed by the irreversible, non-enzymatic addition of glucose to one or both N-terminal valine molecules of the β-globin chain of haemoglobin (Hb). [2] Other

non-enzymatic glycation products of Hb include HbA1a and HbA1b, which are formed by the binding of fructose-1,6-diphosphate and glucose-6-phosphate, respectively. [3] Several conditions, such as haemoglobinopathies, malignancy, haemolysis and anaemia, may cause low or undetectable HbA1c values. [4] Variant Hbs are often associated with spurious HbA1c results, which do not correlate with glycaemic control. Some Hb variants may result in overestimation, underestimation or non-estimation of HbA1c. [5, 6] We present a case of a non-diabetic Nigerian woman with undetectable HbA1c by turbidimetry and high-performance liquid chromatography (HPLC) due to the co-inheritance of two β-globin Hb variants (HbS and HbC).

## Case Description

A 42-year-old woman had HbA1c testing done during a community screening project for diabetes mellitus (DM). The screening was done with the Cobas C311® autoanalyser. HbA1c was undetectable. Fasting plasma glucose (FPG) (3.1 mmol/L) was normal. A fresh specimen was requested, and the HbA1c assay was repeated using the same instrument, but no result was generated. The HbA1c test was repeated by Fluorescence Immunoassay (Ichroma II™, Boditech Med Inc., Chuncheon-si, South Korea), yielding a HbA1c level of 4.5%, which was the analyser's lower limit of detection (reference range: 4.5-6.5%). This raised the suspicion of the presence of an abnormal Hb variant. Haemoglobin electrophoresis conducted on cellulose acetate at pH 8.9 revealed Haemoglobin SC. She was married with four children and had a history of mild-to-moderate anaemia in pregnancy and during confinement with haematocrit (Hct) between 21% and 27%. There was no previous blood transfusion, hospitalisation, painful crisis, or haemolytic crisis.

On further probing, the patient revealed that her Hb phenotype had been tested on three occasions at private laboratories, with conflicting results of HbAS from two of the laboratories and HbAC from the third. To resolve this, the Hb phenotype and variant quantification were freshly done using automated methods: Gazelle Reader (HemexDx Pvt, Mumbai, India), Minicap Analyser (Sebia, Lisses, France), both of which were done at the Department of Haematology and Blood Transfusion, OAUTHC, Ile-Ife and D-10 Analyser (Bio-Rad Laboratories, Hercules, CA, United States of America) which was processed at the Department of Chemical Pathology, University of Benin Teaching Hospital, Benin-City, Nigeria. The Hb phenotype was determined by automated microchip cellulose acetate electrophoresis (Gazelle Reader, HemexDx Pvt, Mumbai, India) and was HbSC disease, with HbS and $HbA_2$/C of 46% and 54%, respectively (Figure 1).

Capillary electrophoresis (Minicap Analyser, Sebia, France) showed $HbA_2$, S, C and F quantifications to be 5.6% (reference value: 1.0 - 3.0%), 47.6%, 40.3% and 6.5% (reference value: 0 - 1.0%), respectively (Figure 2). The HPLC result (D-10 analyser) showed: Unknown, HbA1a, A1b, HbF, $A_0$, $A_2$, HbS, and HbC as 0.8%, 0.9%, 0.2%, 4.3%, 2.1%, 3.8%, 47.3% and 41.4%, respectively. No HbA1c value was obtained (Figure 3).

A full blood count (FBC) showed haematocrit of 28.2%, total white cell count 8.9 x $10^9$/L, neutrophils 59%, and lymphocytes 41%. The peripheral blood film (PBF) showed red cell anisopoikilocytosis, microcytosis, hypochromia and targeting, with normal white blood cells and adequate platelets. The blood group was O Rh D positive, and the serum ferritin level (156 ng/mL) was normal.

*Ethical considerations*

The study was conducted in accordance with the Declaration of Helsinki for experiments involving humans. Ethical approval was obtained from the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife (ERC/2024/11/07). Informed consent was

obtained from the patient for the use of her data in the publication of this report.

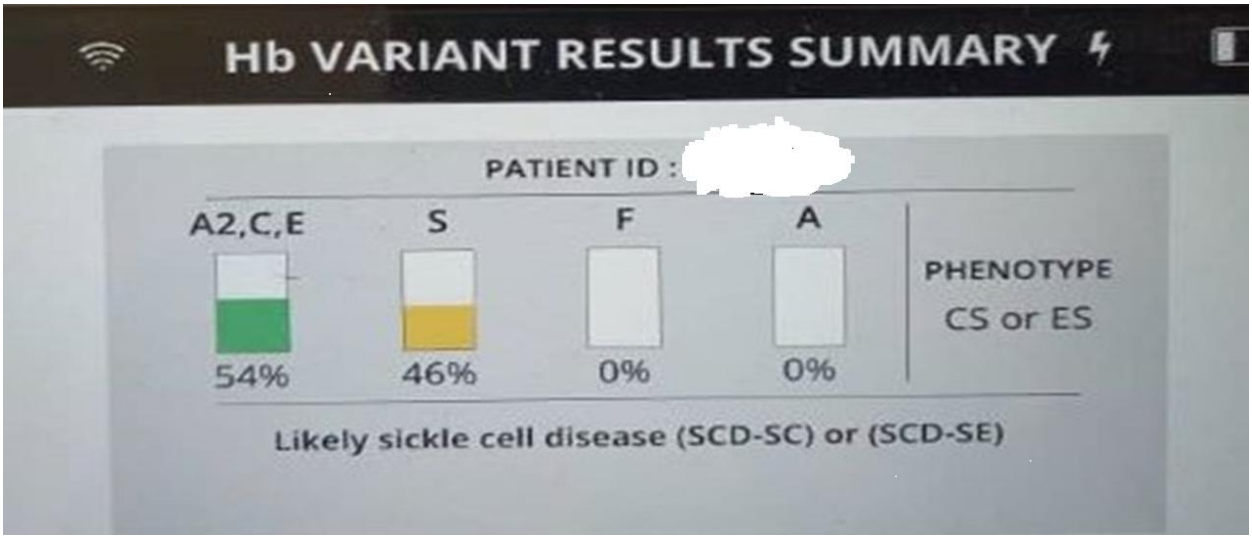
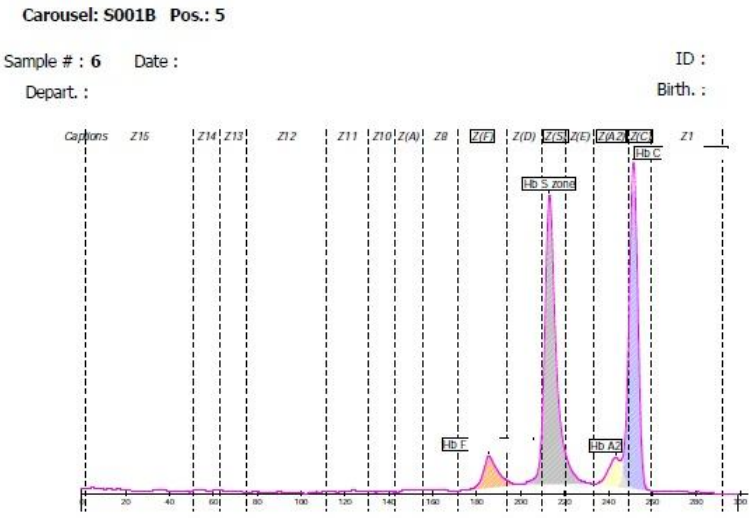



**Figure 1: Haemoglobin variant profile on Gazelle analyser.**
**Haemoglobin variant quantification on Gazelle analyser showing Hb A/C/E - 54%, HbS - 46%, HbF - 0%, HbA - 0%, in keeping with HbSC disease.**




*Haemoglobin Electrophoresis*

| Name | % | Normal Values % |
|---|---|---|
| Hb F | 6.5 | |
| Hb S zone | 47.6 | |
| Hb A2 | 5.6 | |
| Hb C | 40.3 | |

**Figure 2: Haemoglobin variant profile by capillary electrophoresis on Sebia Minicap analyser.**
**Capillary electrophoregram showing the patient's haemoglobin variants from left to right: HbF - 6.5%, HbS - 47.6%, $HbA_2$ - 5.6% and HbC - 40.3%.**

## Discussion

Glycated haemoglobin is the principal subunit of Hb formed by the irreversible non-enzymatic addition of glucose to one or both N-terminal valine molecules of the β-globin chain of Hb. [2] The primary type of haemoglobin found in normal adults is haemoglobin A (HbA). The commonly available assay methods for HbA1c include those that detect structural differences between glyco groups on haemoglobin, such as boronate affinity chromatography and immunoassays, and those that detect charge differences between glycated Hb and non-glycated Hb, such as capillary electrophoresis, cation-exchange HPLC, isoelectric focusing, and direct enzyme assays. [2] All these methods were designed to quantify HbA1c, which constitutes about 80% of total glycated HbA (gHbA). The other non-enzymatic glycation products of Hb include HbA1a and HbA1b, which are formed by the binding of fructose-1,6-diphosphate and glucose-6-phosphate, respectively. [3] Several factors may interfere with the accuracy of HbA1c measurements, such as haemoglobinopathies, malignancy, haemolysis, anaemia, pregnancy, liver cirrhosis, drugs and lipaemia, leading to the generation of spuriously low or elevated HbA1c values. [4]

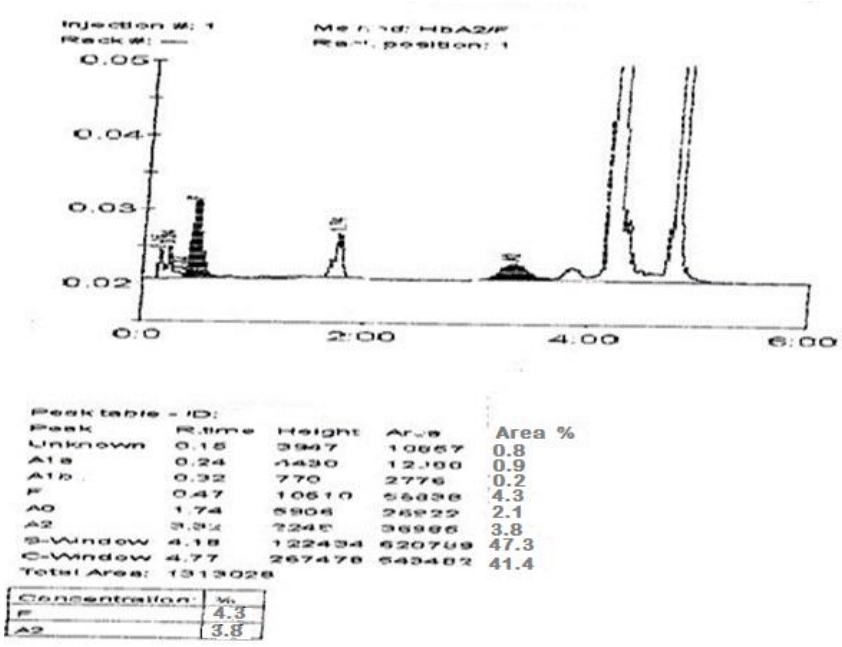



**Figure 3: Haemoglobin variant profile by HPLC.**
**HPLC graph showing peaks for HbA1a - 0.9%, HbA1b - 0.2%, HbF - 4.3%, HbAo - 2.1%, $HbA_2$ - 3.8%, HbS - 47.3% and HbC - 41.4%. HbA1c was not detected.**

Haemoglobinopathies are genetic disorders of Hb. There are two main subgroups: thalassaemias, characterised by reduced synthesis of Hb, and structural haemoglobinopathies, characterised by the synthesis of abnormal Hb variants with altered structure. [7] Haemoglobins S and C are structural variants of normal adult Hb (HbA) resulting from the substitution of glutamic acid at position six of the beta-globin chain by valine and lysine, respectively. Both HbS and HbC are less soluble than HbA. Haemoglobin S polymerises in the deoxygenated state and distorts RBCs into a sickle shape, while HbC forms hexagonal crystals. The inheritance of HbS in the homozygous state (Sickle Cell Anaemia) or in a compound heterozygous state with another abnormal Hb variant like C or D or G (Sickle Cell

Disease) are well known but SCA is the commonest subtype of SCD. Nigeria has the highest prevalence of SCD in the world with a prevalence of about 40% for the HbAS carrier state. [8] The most frequent SCD phenotype is SS, followed by SC.[8] Haemoglobin SC often presents as a milder form of SCD compared to homozygous HbS, but affected individuals are still at risk of severe morbidity. [9]

Many Nigerians are unaware of their Hb phenotype due to a weak policy on newborn and population screening. [8] Also, analytical errors may yield incorrect Hb phenotype results, as observed in the index case, with prior discordant results from private laboratories. In addition, many commercial and government laboratories use only the conventional method of haemoglobin phenotyping by cellulose acetate electrophoresis at alkaline pH only. The interpretation is often based on visual identification of bands, which may be difficult without the use of known controls. Another inherent limitation is that the migration of HbS is similar to that of Haemoglobins D and G. At the same time, HbC migrates similarly to Hbs $A_2$, E and O at alkaline pH, which may require additional electrophoresis at acidic pH, which is not often available in low-resource settings. Thus, many individuals are wrongly labelled, usually as having sickle cell trait (SCT or HbAS) instead of sickle cell disease.

A few individuals with SCD are either asymptomatic or have mild clinical features that are mainly managed outside the hospital and remain undiagnosed. The widespread use of local remedies to manage sickle cell disease among Nigerians could cause underdiagnosis of SCD. These remedies include herbs or food supplements that prevent or alleviate painful crises and improve anaemia in affected individuals. [10] This is the experience of the index patient. Also, late diagnosis is not uncommon in patients with HbSC, such that the diagnosis could be made after age 18 years in about 29% of cases, and delayed up to age 68 years. [11] The relatively asymptomatic nature of the patient may be due to the inhibition of HbS polymerisation by HbC. [12] The absence of symptoms could also be due to co-inheritance of the thalassaemia trait, given the presence of microcytosis, hypochromia, increased HbF, and $HbA_2$ in the absence of iron deficiency (normal serum ferritin), but this could not be further investigated due to technical limitations.

Variant Hbs are often associated with lower HbA1c results, which do not correlate with glycaemic control. Some Hb variants may result in overestimation (HbF), underestimation or non-estimation of HbA1c. [5, 6] The mechanisms by which hemoglobinopathies interfere with HbA1c results include the influence of the binding of glucose to Hb, interactions with chromatography peak measurements, and the increased risk of haemolysis and associated decrease in the life span of red blood cells.[13] The interference of Hb variants with HbA1c assays is method-dependent, with greater interference observed with ion-exchange HPLC. Thus, misleading HbA1c results could be seen with some methods but not with others. Glycated forms of abnormal variants of Hbs, such as S, C and E, are not measured by ion exchange HPLC due to non-elution, resulting in falsely low HbA1c results. [14, 15]

In Southern Brazil, 56.2% of individuals with very low HbA1c measured by HPLC had abnormal Hb variants, while five non-diabetic homozygous HbS patients had HbA1c results of 0%. [4] A previous study showed that African Americans with SCT had significantly lower HbA1c (5.7%) than those without SCT (5.9%), indicating that HbA1c may underestimate previous glycaemia in individuals with SCT. [16] Similarly, African Americans with SCT had lower mean HbA1c than whites, despite higher 2-hour postprandial plasma glucose, further highlighting the limitations of HbA1c measurements in individuals with haemoglobinopathy. [17] Another previous study reported discordant HbA1c results (HPLC) in individuals with Hb variants, such that HbA1c results in individuals with the HbS phenotype

correlated with FPG. In contrast, the HbA1c in patients with HbD, Hb Las Palmas, Hb Louisville, Hb N-Baltimore, or Hb Porto Alegre did not correlate with FPG but rather with the mean glucose concentration in the preceding six to eight weeks, depending on the mean half-life of the RBCs. [5]

The immunoassay techniques commonly used on most automated platforms have less effect and fewer interferences from Hb variants. [5] The immunoturbidimetric assay method, which recognises the epitope within the first six amino acids of the N-terminal β-globin chain, was reported to be susceptible only to rare Hb variants such as Okayama or Graz. [1, 5] The Cobas C311 analyser uses the turbidimetric inhibition immunoassay (TINIA) and could theoretically quantify HbA1c in the presence of haemoglobinopathies like AS, AC and AE. [18] Thus, the presence of either HbS or HbC was not expected to interfere with HbA1c measurements by immunoturbidimetric assays. [5, 18] However, in the index case, the HbA1c could not be quantified on the Cobas C311 even after repeated assays. This could be due to the co-expression of both HbS and HbC. The Ichroma II analyser gave an HbA1c result of 4.5%, which was the analyser's lower limit of detection. These results are in keeping with previous reports of the difficulty in quantification of HbA1c in those who are homozygous or double heterozygous for abnormal haemoglobin variants such as HbS and HbC. [6] The three different methods used to determine the Hb phenotype confirmed that she is a double heterozygote for HbS and HbC. A previous study reported discrepancies in HbA1c results for diabetic patients with haemoglobinopathies, especially those with HbE, on the Cobas C501 analyser, an improved version of the Cobas C311 used for this case. [19]

Also, most of the HbA1c assay methods were validated in the Caucasian population, with predominant HbA1c levels, using a reference value of >6.5% for the diagnosis of DM. [5] This cut-off value did not account for the influence of Hb variants on the assay and results, especially among blacks with DM. As such, there are no recommended cut-off values for HbA1c in individuals with Hb variants or DM found in Africa, India, or Arab countries. This analytical limitation may explain the undetectable result displayed for this case on the C311 and D-10 analysers. The underestimation of HbA1c in individuals with abnormal Hb variants by immunoturbidimetry, HPLC, and immunochromatography makes it challenging to use HbA1c for screening or diagnosis in these individuals. In Port Harcourt, Nigeria, among patients with unknown Hb phenotype, the HbA1c was found to be less accurate for the diagnosis of DM when compared with the Oral Glucose Tolerance Test (OGTT). Diabetes mellitus was diagnosed by HbA1c in 7.8% of patients versus 25.3% by OGTT. [20] This indicates the limitation of the HbA1c as a diagnostic or monitoring tool for DM when the Hb phenotype is unknown. This is compounded by the lack of specific, sensitive platforms such as the Gazelle analyser, HPLC, and capillary electrophoresis for haemoglobinopathy diagnosis in most hospitals in Nigeria. The impact of the high prevalence of haemoglobinopathies in African countries raises questions about the utility of HbA1c for diabetes screening, diagnosis, and monitoring, as its value needs to be reviewed for local clinical use.

## Conclusion

It is essential to ascertain the Hb phenotypes of individuals in populations with a high prevalence of haemoglobinopathies when using HbA1c for DM screening, diagnosis, or monitoring. This will ensure the proper selection of suitable methods for HbA1c analysis or alternative biomarkers. Abnormally low HbA1c levels in individuals with haemoglobinopathies could result in missed DM diagnosis, which may increase the risks of complications of DM. The massive burden of haemoglobinopathies in Nigeria and the rising prevalence of DM in the country necessitate the need for the standardisation of analytical methods. More sensitive tools for Hb phenotyping should be

deployed in Nigerian hospitals to ensure the information is readily available.

**Authors' Contributions:** OTO, AAK and ATA conceived the research and designed the study. OTO, AAK and MAR drafted the manuscript. All the authors revised the manuscript for sound intellectual content and approved the final draft of the manuscript.

**Conflicts of Interest:** None

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