

A Rare Hemoglobin Variant ($\beta 51\text{Pro} \rightarrow \text{His}$) Causing Misleading Measurements of Hemoglobin A_{1c}

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Abstract

Glycated hemoglobin A_{1c} (HbA_{1c}) is considered the standard of care for the testing and monitoring of diabetes. Its ability to accurately reflect glycaemia, however, is imperfect. Hemoglobin variants—mutant forms of hemoglobin caused by genetic variation present in 7% of the population—are known to adversely affect the ability of HbA_{1c} measurement to reflect glycaemic control. We report an illustrative case of a 64-year-old nondiabetic man with a steadily decreasing HbA_{1c} and no symptoms of hypoglycaemia or concerning family history. Preliminary investigative workup returned nothing of significance. Genetic sequencing, however, identified a rare benign hemoglobin variant: a heterozygous missense mutation in the gene encoding the hemoglobin β chain (c.155C > A, p.Pro51His). This variant has been reported only once previously, and the report predates genetic sequence data of the variant. Although this variant had no clinical implications for the patient, it was the cause of falsely low HbA_{1c} levels on high-performance ion-exchange chromatography. This case highlights the importance of considering the effect of hemoglobin variants on the measurement of HbA_{1c}. When available, family history should be carefully considered. Clinicians should suspect hemoglobin variants when HbA_{1c} is too high or low, or discordant with the clinical picture.

Key Words: hemoglobin variant, hemoglobin A_{1c}

Abbreviations: HbA, adult hemoglobin; HbA_{1c}, glycated hemoglobin A_{1c}; HPIEC, high-performance ion-exchange chromatography.

Glycated hemoglobin A_{1c} (HbA_{1c}) is currently considered the standard of care for the testing and monitoring of diabetes and its complications [1]. HbA_{1c} is a glycated form of normal adult hemoglobin (HbA), in which a glucose is attached to the N-terminal valine of each β chain in the hemoglobin tetramer [2, 3]. Owing to the lifespan of erythrocytes, HbA_{1c} correlates with blood glucose over the preceding 120 days. Levels of HbA_{1c} can be measured by many methods, and the choice of method is often center specific. Methodologies include enzyme assays and immunoassays, affinity chromatography, capillary electrophoresis, high-performance liquid chromatography (HPIEC). Regardless of methodology, measurement of HbA_{1c} as a marker of diabetes control has distinct advantages: It is known to capture chronic hyperglycemia better than other measures, and is better associated with complications [4].

The measurement of HbA_{1c}, however, is imperfect. Because HbA_{1c} is based on the turnover of erythrocytes and their glycation, its accuracy can be adversely affected by conditions or states that affect these processes. Thus, comorbidities are well known to reduce the utility of using HbA_{1c} to diagnose and monitor diabetes [4]. Iron-deficiency anemia, asplenia,

and chronic alcohol consumption cause increased levels of HbA_{1c}, whereas blood loss, transfusions, and pregnancy can cause falsely decreased levels of HbA_{1c} [4].

Hemoglobin variants can also impede the accurate measurement of HbA_{1c} [4–6]. HbA is composed of 4 subunits, 2 α chains, encoded by genes on chromosome 16 (*HBA1* and *HBA2*), and 2 β chains, encoded by a gene on chromosome 11 (*HBB*). Genetic variants can occur in any of these genes. Most variant forms of hemoglobin, however, are the result of a missense variant leading to a single amino acid substitution in the β chain [2, 3]. Seven percent of the population has a variant form of HbA [2, 3]. The clinical presentation of these variants is heterogeneous: Variant carriers have presentations ranging from hemolytic anemia and reticulocytosis to erythrocytosis [3]. Many variants, however, are clinically silent, with carriers being asymptomatic [3]. Even clinically silent hemoglobin variants, however, can affect measurement of HbA_{1c}, with some methodologies more affected than others [4]. Depending on the method and the hemoglobin variant, HbA_{1c} results can be falsely low or high, potentially impeding the accurate monitoring of diabetes control, or even leading to inappropriate diagnoses or the unnecessary treatment of unaffected patients.

To illustrate the effects of measuring HbA_{1c} in the setting of hemoglobin variants, we present a case of a clinically well individual with a measured low HbA_{1c} due to a rare variant of hemoglobin, as well as the confirmed genetic etiology.

Case Report

A 64-year-old man with a body mass index of 25.1 was referred to Endocrinology with low HbA_{1c}, as measured by HPIEC, that had been decreasing over the preceding 7 years (Fig. 1). Aside from occasional dizziness, he had no classic symptoms of hypoglycemia—fasting, overnight, nor postprandial. He ate regular meals, with no eating overnight or unusual dietary patterns. He had essentially stable weight, no history of liver disease, blood dyscrasias, congenital syndromes, nor a history of bleeding or transfusions. His past medical history was significant only for well-controlled psor-

iasis and immunoglobulin A nephropathy with proteinuria. He took ramipril 2.5 mg daily and used a topical steroid cream as needed. Early in his presentation he reported no remarkable family history. He worked in the legal field, drank 2 to 3 beers per day, and had 4 grown healthy children. With the exception of being overweight as per his previously recorded body mass index, his exam was normal.

Initial investigations and imaging were largely unremarkable (Table 1). In contrast to the low HbA_{1c}, random and fasting glucose were both normal (5.1 mmol/L). At follow-up visits the patient's random glucose measurements remained between 5.1 and 5.2 mmol/L. The patient had an appropriately normal fasting glucose, insulin, and C-peptide level (703 pmol/L).

At the time of his visit, HbA_{1c} was deemed not reportable. The HbA_{1c} assays, including those measurements previously taken in the community, were performed locally on the Variant II Turbo (Bio-Rad Laboratories). This is based on an HPIEC that has no interferences from hemoglobin S, C, D, or E variants. The presence of β -thalassemia trait, fetal hemoglobin (up to 25%), and carbamylated hemoglobin and labile A_{1c} also do not affect the accurate reporting of HbA_{1c}. When calculated using a fructosamine assay, the HbA_{1c} was 4.9% (fructosamine = 196 μ mol/L; A_{1c} (%) = 0.017 \times fructosamine + 1.61 as per Cohen et al 2003 [7]), which remains discordant with HbA_{1c} measured by HPIEC, despite a possible lower glycosylation of fructosamine secondary to our patient's ongoing proteinuria. Thus, hemoglobin electrophoresis was undertaken to investigate explanatory hemoglobin variants. Initial hemoglobin electrophoresis was normal: HbA 97.1%

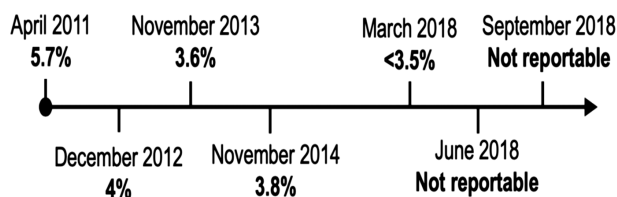


Figure 1. A_{1c} timeline from 2011 to 2018. A_{1c} deemed “not reportable” by the laboratory in June and September 2018 because of an identified abnormal hemoglobin peak affecting the appropriate measurement of A_{1c}.

Table 1. Relevant investigations and results. Investigations were undertaken June 2018 unless otherwise specified

Investigation	Result
Hemoglobin A _{1c}	Not reportable
Glucose	5.1 mmol/L, random 5.1 mmol/L, fasting
Insulin	48 pmol/L (< 120 pmol/L)
C-peptide	703 pmol/L (260-1730 pmol/L)
CBC	HgB 148 g/L Hct 0.441 RDW MCV normal WBC 5.39 $\times 10^9$ /L Plt 226 $\times 10^9$ /L
Peripheral blood smear	Normal
Hemoglobin electrophoresis	No hemoglobin variant detected, no thalassemia
TSH	4.84 mIU/L (0.35-4.3 mIU/L)
8 am serum cortisol	434 nmol/L
Fructosamine (calculated HbA _{1c})	196 μ mol/L (205-285 μ mol/L), September 2018 [A _{1c} (%) = 0.017 \times fructosamine + 1.61] (calculated HbA _{1c} = 4.9%; measured HbA _{1c} not reportable, per laboratory)
AST, ALT, ALP, GGT	Normal
Urine protein	0.3 g/L, March 2021
Bilirubin, albumin, INR	Normal
Creatinine (GFR)	91 μ mol/L (77 mL/min/1.73 m ²)
Abdominal ultrasound	Normal liver, normal pancreas, normal kidneys normal spleen (10 cm), December 2018

Abbreviations: ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; CBC, complete blood count; GFR, glomerular filtration rate; GGT, γ -glutamyltransferase; HbA_{1c}, glycated hemoglobin A_{1c}; Hct, hematocrit; HgB, hemoglobin; INR, international normalized ratio; MCV, mean corpuscular volume; Plt, platelets; RDW, red blood cell distribution width; TSH, thyrotropin; WBC, white blood cell count.

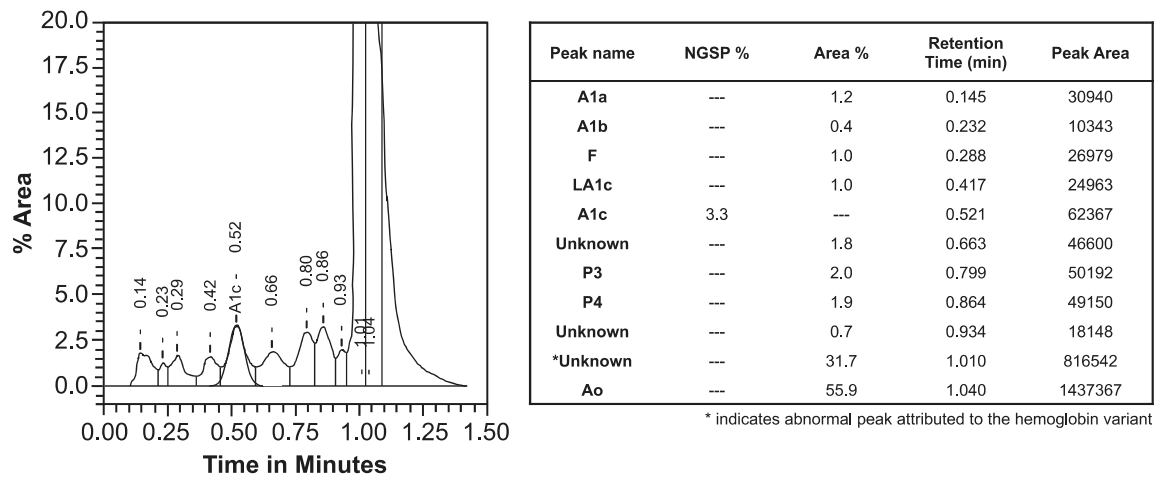


Figure 2. High-performance ion exchange chromatography results for the determination of hemoglobin A_{1c}. This graph was generated by the analyzer and is shown as is, with the table transcribed verbatim. % Area is represented by the y-axis, Time in minutes by the x-axis, and the abnormal peak (subsequently identified as the Hemoglobin North Manchester peak) is identified by the asterisk.

and HbA₂ 2.9%. Ultimately, on repeat analysis of HbA_{1c}, an abnormal hemoglobin peak on HPIEC was noted by the laboratory during manual review (Fig. 2).

In light of the abnormal heterozygotic peak on his HbA_{1c} assay and normal hematologic workup including electrophoresis, genetic sequencing was undertaken. Sequencing identified a heterozygotic variant in *HBB*, the gene encoding the hemoglobin β chain. The single-nucleotide variant (NM_000518.4[*HBB*]:c.155C > A, NC_000011.10:g.5226737G > T) is a missense variant at coding position 155 (cysteine to adenosine), resulting in a single amino acid substitution, from a proline to a histidine residue. This substitution results in a β chain with increased mass, creating an abnormal additional peak (and smaller normal β -chain peak) when analyzed by mass spectrometry [8]. It has been reported once previously as the Hb North Manchester Mutation [8]; however, this was by amino acid sequence only. In our patient, the laboratory reported the hemoglobin variant resulting from the detected nucleotide variant as benign. Carriers of benign variants are expected to have a normal phenotype, as the variant is not predicted to have any clinical effects. Of note, however, the variant is exceedingly rare: The genome aggregation database (<https://gnomad.broadinstitute.org>) reports only one instance of the variant, giving an allele frequency of 7×10^{-6} . Among other pieces of evidence, allele frequency is used to determine the pathogenicity of genetic variants, with higher-frequency variants being less likely to cause penetrant disease [9].

After receiving the genetic diagnosis, our patient continued to be asymptomatic, including normal point-of-care glucose monitoring. He was counseled on alternative methods of screening for diabetes including fasting glucose levels and oral glucose tolerance tests, which are not affected by the genetic variant. It was further determined that he had a sibling with a similar abnormal hemoglobin peak on a recent HbA_{1c}, although further genetic testing could not be pursued in this individual.

Discussion

This case is unique because it is one of the first to demonstrate the nucleotide variant underlying the Hb North

Manchester in a patient. Furthermore, it is the first report of the variant in North America, as well as the first in a patient without diabetes: To date, only the original report of the Hb North Manchester variant in 2 related patients in the United Kingdom and a 42-year-old Chinese man with the variant have been published [8, 10]. Both of these previously reported cases involved individuals with diabetes with discordant HbA_{1c} and glucose measurements affected by the heterozygotic presence of the variant. Of note, a different variant at the same site, where an arginine replaces the proline, has been described as Hemoglobin Willamette [8]. Although most patients described with the Hemoglobin Willamette variant are asymptomatic, Barbosa et al report [11] on a patient who presented with a symptomatic refractory microcytic anemia. Their patient's laboratory tests showed a hemoglobin of 84 g/L, hematocrit of 23.4%, mean corpuscular volume of 75.0 fL, reticulocytes count of 4.8%, normal iron studies, and a peripheral smear featuring anisochromia, microcytosis, and polychromasia. Importantly, to date, no homozygous carriers of the Hb North Manchester variant have been reported. Taken together with the possibility of a clinical phenotype in a heterozygous carrier of a variant at this site, it is certainly possible that this sequence is conserved for functional reasons, and that a homozygous variant at this site could be pathogenic.

Hemoglobinopathies can affect HbA_{1c} values in 3 ways: by influencing the binding of glucose to hemoglobin, by decreasing the lifespan of red blood cells, or by affecting chromatography peak measurements [12]. In the case of chromatography, coelution of the hemoglobin variant with HbA_{1c} will cause gross overestimation of HbA_{1c}, while coelution of the hemoglobin variant with HbA₀, with resolution of the glycated hemoglobin variant from HbA_{1c}, will underestimate the HbA_{1c} results, as seen in this case. Wiener et al [8] demonstrated that the Hb North Manchester amino acid substitution was present in approximately half the total hemoglobin β chains. In turn, HbA_{1c} measured by HPIEC was half that of the value as measured by affinity chromatography. In our patient, the cause of the gradual decrease in HbA_{1c} over time, however, remains unknown. It could be attributed to factors known to lower HbA_{1c}, [4] but it could be due to mosaicism that led to greater proportions of total hemoglobin represented by mu-

tated chains over time. This could be further tested through molecular hemoglobin analysis of the patient's sibling with reported low HbA_{1c}. Unfortunately, the sibling did not consent to further investigations or genetic confirmation of the variant.

This case is important in that it demonstrates that variant hemoglobins, even forms that do not cause clinical disease, can cause erroneous measures of HbA_{1c}. In this case, the patient was a carrier of a benign variant hemoglobin, Hb North Manchester, that led to falsely low HbA_{1c} levels when measured by HPIEC. This highlights an important flaw in using HbA_{1c} as a marker for diabetes, and should prompt clinicians to consider hemoglobin variants when HbA_{1c} is outside the normal range and not in keeping with the clinical picture. Furthermore, it is plausible that a high HbA_{1c} level could be masked by such a hemoglobin variant, depending on the method of measurement, leading to a false assessment of well-controlled diabetes and a missed opportunity for subsequent management.

This case also demonstrates that just because a variant is “benign”—whether that be a genetic variant or a hemoglobin variant—does not mean it will have no clinical effect. In this case, although the hemoglobin variant was not disease-causing, it did cause erroneous HbA_{1c} levels. In turn, this led to many subsequent investigations—which, in itself, is not entirely benign.

Conclusions

This case highlights the pitfalls of measuring HbA_{1c}, which can be erroneous in the setting of a variant hemoglobin. Even if benign, these variants can have a significant clinical effect that warrants careful consideration. Where HbA_{1c} is used, clinicians should be mindful of the effect of hemoglobin variants on the accuracy of the measure. Variants should be suspected when HbA_{1c} is higher than expected relative to ambient glycemia, below the nondiabetes reference range, or discordant with the clinical picture [13]. Furthermore, chromatography traces should be examined closely for anomalies if a hemoglobin variant is being considered [6]. Family history should be carefully examined because of the potential for hemoglobin variants to cause falsely low or even falsely normal HbA_{1c} readings. In light of these pitfalls, we caution against the exclusive use of HbA_{1c} for screening and monitoring of diabetes. Where indicated, fasting glucose or oral glucose tolerance tests can be used as alternative screening methods for diabetes and assessment of its control.

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Disclosures

The authors have nothing to disclose.

Data Availability

Data sharing is not applicable to this article because no data sets were generated or analyzed during the present study.

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