Incidental detection of a rare hemoglobin variant (Hemoglobin N Seattle) leading to undetectable levels of HbA1c in a diabetic female: a case report

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Abstract

Glycosylated hemoglobin (GHb) is routinely used to monitor glycemic control over past 2-3 months in diabetics. As per the recommendations of the American Association of Clinical Endocrinologist, 2007 values should be maintained below 7% to prevent the risk of chronic complications. We report a case of a 55-year old female patient with spuriously low HbA1c values by high-performance liquid chromatography. Suspecting the presence of any abnormal hemoglobin, capillary zone electrophoresis was done which identified the presence of Hb variant corresponding to -Hb N Seattle. Our case highlights that clinical laboratories should be aware of limitations of their HbA1c assay methods as well as rule out any possible interfering Hb variants.

Introduction

Glycated haemoglobin (GHb), measured as HbA1c, is routinely done to assess long-term glycemic control in diabetics. It provides an index of average blood glucose levels over past two to four months.¹ However, many hemoglobin variants interfere with glycated hemoglobin estimation.² These variants may lead to either erroneous high or low HbA1c levels. Most of the rare hemoglobin variants that are clinically silent are often revealed during evaluation of discordant HbA1c levels. The influence of hemoglobin variants on HbA1c is dependent on type of method used and is found to be higher when ion exchange high-performance liquid chromatography (HPLC) is used.^{2,3} Here, we report a rare Hemoglobin variant *Hb N Seattle* (HBB:c.184A>G) that lead to aberrantly low HbA1c values in a known diabetic, and also discuss factors that may affect HbA1c measurements.

Case Report

A 55-year old female a known hypertensive, presented with increased frequency of urination and weight loss. Routine blood investigation showed increased random blood sugar levels. On examination she had no pallor, icterus or organomegaly. Complete blood count, lipid profile and fasting blood sugar were done (Table 1). HbA1c estimation was done by HPLC using D10 BioRad system. The HbA1c was not detectable and revealed a large peak in the labile A1c window (28.5%) with a retention time of 0.68 sec (Figure 1). No previous HbA1c reports were available with the patient. Presence of any interfering hemoglobin variant was suspected. Further investigations were done including capillary zone electrophoresis (Sebia minicap) to detect any abnormal hemoglobin variant (Figure 2). Hemoglobin electrophoresis showed decreased levels of adult hemoglobin A (69.1%) and an abnormal peak (29%) in zone 14 corresponding probably to Hb N Seattle. Family screening and molecular studies for confirmation of the variant was advised but the patient was lost to follow up.

Discussion

Hemoglobin N Seattle is a rare variant hemoglobin first discovered in a Seattle man of African ancestry. The structure of hemoglobin N Seattle was found to be substitution of a glutamic acid residue for the lysine residue in position 61 of the β -chain [$\beta 61(E5)Lys >Glu$].⁴ It is clinically silent in heterozygotes and is seen in ethnic background, mostly black males.

According to the American Diabetes Association, 2010 a cut off value at 7% of GHb values should be maintained to avoid chronic complications.¹ GHb is formed by a two-step reaction. First reaction is a rapid reversible one where a labile aldimine or Schiff base is formed. Subsequently the aldimine undergoes Amadori rearrangement and is converted to a stable ketoamine, glycosylated hemoglobin.⁵ According to International Federation Of Clinical Chemistry Working Group, HbA1c is defined as hemoglobin A that is irreversibly glycosylated at one or both N terminal valines of the β chains of tetrameric hemoglobin molecule, including hemoglobin that may also be glycosylated on lysine residues.⁵

The level of HbA1c is proportional to average glucose concentration as well as the life span of the red blood cell in the circulation. Both patient and laboratory factors can result in misleading HbA1c values.

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Erroneously low HbA1c is seen in conditions with increased cell turnover and reduced life span of the red blood cells like active bleeding, hemolytic disease, haemoglobinopathies and myelodysplastic syndromes.³ In renal failure and uraemia, high levels of carbamylated haemoglobin, may lead to aberrantly high HbA1c. Spuriously elevated HbA1c measurements by HPLC is seen when red blood cell turnover is low, resulting in higher proportions of older red blood cells in circulation, such as in iron, B12 or folate deficiencies.

Suspicion arises when there is any discrepancy between a patient's blood glucose levels and laboratory measured HbA1c. HbA1c of more than 15%, or a significant change in a patient's HbA1c together with a change in laboratory HbA1c assay method alerts for careful interpretation of results. One can repeat the HbA1c measurement with a different assay method to observe for discrepancies. Further investigation like complete blood count, reticulocyte count, serum haptoglobin and Hb electrophoresis should be done when relevant.

Conclusions

It is of utmost importance that HbA1c results should not be interpreted in isolation but should always consider the patient's medical history and related laboratory findings. In addition, efforts should be made to identify the Hb variant, and alternative HbA1c methods that are free of the interference should be used. Lastly, each laboratory should know as well as convey their clinicians when need the limitations for the var-

Peak table - ID: 02604492

Peak	RetTime	Height	Area	Area %
A1a	0.21	43027	131662	7.3
A1b	0.34	11400	57808	3.2
LA1d/CHb-1	0.68	36449	516222	28.5
P3	1.38	30107	124961	6.9
A0	1.48	272164	977708	54.1

Total Area: 1808361

Concentration:	%
—	0.0

Figure 1. HPLC (biorad D10) showing undetectable HbA1c.

Figure 2. Capillary zone electrophoresis showing abnormal peak in zone 14 corresponding to hemoglobin N Seattle.

Table 1. Laboratory parameters (hemogram and biochemical).

Hemoglobin	16.0 mg%
PCV	51.5%
TRBC	4.57×10^3
MCV	92.6 fL
MCH	29 pg
MCHC	31.3 mg/dL
TWBC (total WBC count)	$10.69 \times 10^3/\mu\text{L}$
TPC (total platelet count)	$430 \times 10^3/\mu\text{L}$
FBS (Fasting blood sugar)	354 mg/dL
PPBS (postprandial blood sugar)	557 mg/dL
Serum triglyceride	265 mg/dL
Total cholesterol	242 mg/dL
LDL	139 mg/dL
VLDL	53 mg/dL

ious methods used to measure HbA1c.

This case report highlights how a spuriously low HbA1c level lead to detection of a very rare hemoglobin variant corresponding to *N Seattle*, which would have missed detection as it is clinically silent.

References

1. American Diabetes Association. Standards of medical care in diabetes - 2010. *Diabetes Care* 2010;33:S11-61.
2. Sofronescu A-G, Williams LM, Dorinda M, et al. Unexpected hemoglobin A1c results. *Clin Chem* 2011;57:153-7.
3. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem* 2001;47:153-63.
4. Jones RT, Brimhall B, Huehns ER, Motulsky AG. Structural characterization of hemoglobin-N-Seattle. *Biochim Biophys Acta* 1968;154:278-83.
5. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. *Tietz textbook of clinical chemistry*, 3rd ed. Philadelphia: WB Saunders Co.; 1999. pp 790-6.