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

Sarita Pradhan,1 Sima Chauhan,1 Priyanka Samal2
1Department of Pathology, Siksha ‘O’ Anusandhan University, Bhubaneswar; 2Department of Clinical Hematology, IMS&SUM Hospital, Bhubaneswar, India

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 [β61(ES)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 aldime or Schiff base is formed. Subsequently the aldime undergoes Amadori rearrangement and is converted to a stable ketoamine, glycosylated hemoglobin. 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 values of the β chains of tetrameric hemoglobin molecule, including hemoglobin that may also be glycated 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.

Correspondence: Sarita Pradhan, Department of Pathology, Siksha ‘O’ Anusandhan University, Bhubaneswar-751030, Odisha, India.
E-mail: saritapradhan@soauniversity.ac.in

Key words: Hemoglobinopathies; HbA1c.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 2 March 2016. Revision received: 28 October 2016. Accepted for publication: 27 December 2016.

This work is licensed under a Creative Commons Attribution 4.0 License (by-nc 4.0).

©Copyright S. Pradhan et al., 2017
Licensee PAGEPress, Italy
Thalassemia Reports 2017; 7:5860

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.1 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-

[Thalassemia Reports 2017; 7:5860]
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