Brief Communication

Hemoglobin E disease and glycosylated hemoglobin

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ABSTRACT

Glycosylated hemoglobin (HbA1C) is a routinely measured parameter to monitor long-term glycemic control in people with diabetes mellitus. The presence of hemoglobin (Hb) variants can affect the accuracy of HbA1C methods. Hb E variant is the most common Hb variant in South-east Asia and North-east India. In the presence of Hb E, HbA1C may not be detectable by ion-exchange chromatography (high-pressure liquid chromatography), but may be estimated by immunoassay technique and boronate affinity chromatography. However, the result may be underestimated when correlated with plasma glucose and serum fructosamine levels. Clinicians should be aware of this limitation of HbA1C estimation in patients with Hb E and other Hb variants.

Key words: Glycosylated hemoglobin, hemoglobin E variant, interference, North-east India

Introduction

Glycosylated hemoglobin (HbA1C) is a biochemical marker that is used to monitor the long-term glycemic control and assess the risk of developing complications.[1] The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study demonstrated that risks for complications are related directly to glycemic control, as measured by HbA1C.[2] The National Glycohemoglobin Standardization Program standardizes HbA1C results so that clinical laboratory results are comparable with those reported by the DCCT. Current American Diabetes Association guidelines recommend that HbA1C <7% is a reasonable goal for many nonpregnant adults. A1C <6.5% may be appropriate for those without significant rise of hypoglycemia and A1C <8% may be appropriate for patients with history of hypoglycemia, limited life expectancy or those with longstanding diabetes and vascular complications.[3] According to American Association of Clinical Endocrinologists/American College of Endocrinology Diabetes Guidelines, HbA1C goal is <6.5% in general, closer to normal for healthy and less stringent for "less healthy" individuals.[4] Hence, HbA1C determination becomes an integral part of diabetes care.

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Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.163211

Challenges in glycosylated hemoglobin estimation

HbA1C estimation may be affected by a variety of genetic, hematologic and disease-related factors [Table 1]. The important factors affecting HbA1C levels are hemoglobinopathies, certain anemias, certain drugs, and disorders associated with accelerated erythrocyte turnover such as malaria.[5] Approximately, 7% of the world's population carries a hemoglobin (Hb) variant, making these variants one of the most common monogenetic diseases and a major health issue worldwide.[6] Almost a thousand Hb variants have been identified, the four most common worldwide are Hb S, Hb E, Hb C, and Hb D, in the order of decreasing prevalence.[7] The Hb E variant is

Cite this article as: Yedla N, Kuchay MS, Mithal A. Hemoglobin E disease and glycosylated hemoglobin. Indian J Endocr Metab 2015;19:683-5.

extremely common in South-east Asia (Thailand, Myanmar, Cambodia, and Vietnam), equaling Hb A in frequency in some areas. In certain areas of North-east India, carrier rates reach up to 60% of the population.[8] Clinicians should bear in mind that these Hb variants are common among the general population, and the accuracy of several HbA1C methods can be affected adversely by the presence of Hb variants [Table 1].[9]

Hemoglobin E disease

Hb E contains a substitution of lysine for glutamic acid at position 26 of the β chain. Hb E is found primarily in people from South-east Asia and North-east India and is relatively uncommon in other parts of the world.[8] Subjects with homozygous E disease and heterozygous E trait are usually completely asymptomatic. Thus, a physician may be unaware that their patient with diabetes has Hb E disease or Hb E trait, and their HbA1C result may be incorrect.

Clinical vignette

To illustrate this, a 50-year-old male, resident of Guwahati, Assam (North-east part of India), known case of type 2 diabetes mellitus for last 8 years, presented to our Out-Patient Department for the Management of Diabetes Mellitus. He had osmotic symptoms in the form of polyuria and polydipsia at presentation. His physical examination revealed no abnormality except for mild splenomegaly. Laboratory parameters revealed fasting plasma glucose of 240 mg/dL and 320 mg/dL 2-h after breakfast. The HbA1C was not readable by ion-exchange high-pressure liquid chromatography (HPLC) and remained undetected on a repeat test from another laboratory using ion-exchange HPLC. Hemoglobinopathy was suspected as a probable cause, as the patient was from North-east area of India, where this condition is relatively more common.[8] Hb electrophoresis was ordered which revealed Hb A of 14.8% (normal range: 94.3–98.5%), Hb E of 82.8% (normal range: 0.00%) and Hb F of 2.4% (normal range: 0–2%). HbA1C could not be detected as Hb A_2, and HbA1C co-eluted at the same time and could not be separated. Hb E disease was diagnosed (homozygous state). Then HbA1C was measured using immunoassay technique (point-of-care apparatus) and was found to be 6.4%. However, with the plasma glucose readings that were obtained, expected HbA1C was higher. Serum fructosamine was 395 μmol/L (normal range: 202–282 μmol/L) that was corresponding with high glycemia and not with the reported HbA1C level. Serum Hb level was found to be 10.2 g/dL accounting for mild anemia. He has a 16-year-old daughter with a history of low Hb (Hb = 8.8–10.5 g/dL). Hb electrophoresis revealed Hb E disease in her as well.

Table 1: Factors that affect HbA1C measurement

Erythropoiesis	Increased HbA1C: Iron deficiency, Vitamin B12 deficiency, decreased erythropoiesis Decreased HbA1C: Therapy with erythropoietin, iron and Vitamin B12, reticulocytosis, chronic liver disease
Altered hemoglobin	Increase or decrease HbA1C: Genetic or chemical alterations in hemoglobin: Hemoglobinopathies, Hb F, methemoglobin
Glycation	Increased HbA1C: Alcoholism, chronic kidney disease Decreased HbA1C: Aspirin (small doses), certain hemoglobinopathies, Vitamin C, Vitamin E
Erythrocyte destruction	Increased HbA1C: Increased red cell life span; splenectomy Decreased HbA1C: Shortened red cell life span; splenomegaly, certain hemoglobinopathies, rheumatoid arthritis and drugs like antiretrovirals, ribavirin, and dapsone
Assays	Increased HbA1C: Increased bilirubin, carbamylated hemoglobin, aspirin (large doses), alcoholism Decreased HbA1C: Increased triglycerides Variable HbA1C: Hemoglobinopathies

HbA1C: Glycosylated hemoglobin, Hb F: Fetal hemoglobin

Choice of glycosylated hemoglobin estimation method

There are four techniques of HbA1C measurement [Table 2]: (1) Immunoassay technique: It specifically measures HbA1C. It uses an antibody that recognizes the structure of the N-terminal glycated amino acids, usually the first 4–10 amino acids on the Hb β chain. (2) Ion-exchange HPLC: This method calculates HbA1C by separating it from other Hbs based on differences in ionic charges. (3) Boronate affinity HPLC: This method utilizes m- aminophenylboronic acid that specifically reacts with the cis-diol groups of glucose bound Hb. These measures

Table 2: Principle, advantages and disadvantages of various HbA1C assays

Method	Principle	Advantages	Disadvantages
Immunoassay technique	Antibody binds to glucose and between 4 and 10 N-terminal amino acids on β chain	Relatively easy to implement Least affected by Hb E	Affected by hemoglobinopathies with altered amino acids on binding sites
Ion exchange chromatography	HbA1C is separated from other hemoglobins because of differences in ionic charges	Hemoglobin variants can be detected from chromograms Measurements with great precision	Variable interference from hemoglobinopathies
Boronate affinity	Glucose binds to m-Aminophenylboronic acid	Minimal interference from hemoglobinopathies	Measures not only glycation of N-terminal valine on β chain but also β chains glycated at other sites and glycated α chains
Enzymatic method	An enzyme that specifically cleaves the N-terminal valine	Simple technique	Interference from hemoglobinopathies

Hb E: Hemoglobin E, HbA1C: Glycosylated hemoglobin

total glycosylated Hb that includes HbA1C, as well as Hb glycated at other sites as well. This estimate is supposed to demonstrate the least interference due to Hb Variants. (4) Enzymatic method: It measures HbA1C by using an enzyme that specifically cleaves the N-terminal valine.

Hemoglobin E and glycosylated hemoglobin

Hb E is one of the common Hb mutations, resulting in disorders varying from mild to severe disease. Since Hb is inherited from both parents, when one Hb E gene is passed on (heterozygous), Hb E trait results. It is a clinically insignificant disease. If both genes transferred have the mutation, then Hb E disease results (homozygous condition). In such cases, the Hb A is very low, with around 80% Hb being Hb E itself. These people have mild anemia and a slightly enlarged spleen as seen in our patient.

Hb E disease usually does not affect the immunoassay technique of HbA1C estimation, as the mutation tends to be farther away on the β chain than 4–10 amino acids. However, it does interfere with the ion-exchange HPLC method, as the mutation tends to alter the ionic charges on the Hb.[10,11] Since, boronate affinity technique calculated total glycosylated Hb by separating the glycated and nonglycated Hb, as a general assumption it should not affect HbA1C measurement.[7,12] But the measured HbA1C could be an underestimate.

Conclusion

In summary, Hb E variant, which is common in the North-eastern part of India, can interfere with the HbA1C measurement depending on the type of assay used.[13] Even if HbA1C is detectable by immunoassay, it is often an underestimate. A different technique, which might not affect the HbA1C, should be tried.[14] Hb electrophoresis must be done to look for hemoglobinopathies in such cases. It is important to consider the risks of inappropriate HbA1C measurements in persons with diabetes, and its impact on treatment. Any result that does not fit the clinical picture should be investigated further.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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