

Case Report

Hemoglobin E: a potential interferent in measurement of glycosylated hemoglobin

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ABSTRACT

Glycosylated hemoglobin (HbA1C) is a routinely measured parameter to monitor long term glycemic control in patients with diabetes mellitus. There are many potential interferents which can affect measurement of HbA1C by high performance liquid chromatography (HPLC). Variant hemoglobins, especially, are a common source of confusion and errors in HbA1C measurement. Authors present an interesting case of Hb E variant (undiagnosed hitherto) which came to attention when the machine repeatedly failed to give Hb A1C levels. Hb E is the commonest Hb variant in North East India. In the presence of Hb E, HbA1C may not be detected by ion exchange chromatography as both hemoglobin's co-elute together, thereby causing errors. In such cases, the clinician may resort to subcutaneous sugar monitoring as an alternate or if required, Hb A1C measurement may be done by other techniques like immunoassay technique or boronated affinity chromatography. The laboratory staff and clinicians, both, should be aware of this limitation of HbA1C estimation in patients with HbE and other Hb variants.

Keywords: Glycosylated hemoglobin, Hemoglobin E variant, High performance liquid chromatography, Interference

INTRODUCTION

Glycosylated hemoglobin (HbA1C) is a biochemical marker that is used to monitor the long-term glycemic control in patients of diabetes mellitus.¹ The diabetic control and complication trial (DCCT) and the United Kingdom prospective diabetes study demonstrated the risks for complications are related directly to glycemic control. Hence, HbA1C levels are also used to assess the risk of developing various complications in this population.² Current American diabetic association guidelines recommend HbA1C <7% as a reasonable goal for diabetic adults on long term follow up of disease. All this makes HbA1C determination becomes an integral part of diabetic care.³

Challenges in glycosylated hemoglobin (HbA1C) estimation-HbA1C estimation by HPLC may be affected

by a variety of genetic, hematologic and disease related factor. Yedla summarizes all the common potential interfering factors in determination of HbA1C as per Table 1 which mainly include hemolytic anemias and drugs.^{4,5} Approximately, 7% of world's population carries an abnormal hemoglobin (Hb) variant, making these variants one of the common and major potential interferent.⁶

This abnormal hemoglobin co-elutes or mask the elution of HbA1C in HPLC technique thereby leading to errors in its measurement. Clinician should bear in mind that the accuracy of several HbA1C methods can be affected adversely by the presence of Hb variants.

Here, authors present a case of a serving soldier, on regular follow-up for diabetes, whose repeated samples were sent for HbA1c measurement and result was given

as “not recordable” by the machine every time. This led the laboratory to suspect an underlying interferent which was then pursued and an underlying hemoglobinopathy was revealed.

CASE REPORT

A 49 years old serving soldier, native of Manipur, had been under regular follow up for hypertension, diabetes mellitus and non-alcoholic steatohepatitis for few years. His annual endocrinal review showed patient to be asymptomatic, drug compliant and free of any emerging complications. His general and systemic examination was within normal limits. Basic investigations showed Hb-14.2 g/dl, TLC-6,700/cmm DLC-P58% 32% M8% E2% platelet count of 220 x 10⁹/l, ESR of 18 mm in first hour, prothrombin time of 12.5 sec, activated prothrombin count of 28 sec. Urine and stool routine examination was within normal limit. Biochemical parameters were also within normal limits with good glycemic control. (sugar fasting - 102 mg/dl post prandial 140 mg/dL - bilirubin-0.3 mg/dl - AST- 35 IU/l ALT - 55 IU/l BUN - 11 mg/dl creatinine - 0.85 mg/dl total proteins - 6.68 gm/dl potassium - 3.90 meq/l sodium - 132 meq/l). However, the sample sent for HbA1C could not give values on HPLC machine (D10 BIORAD laboratories, HPLC) run on glyated hemoglobin mode. Suspecting some preanalytical error/ clot in sample, a repeat sample was asked for which again showed Hb A1C to be not recordable. Subsequently, fresh samples of the patient were run on machine on two more occasions which yielded same results. At this point, the patient was called for detailed history and further evaluations. Perusal of documents revealed that though the hemoglobin values were within normal limits, the red cell indices were pointing towards a possible hemoglobinopathy (Hb - 14.2 gm/dl, TRBC - 6.93×10⁶ /microl MCV - 58.6 fL MCH - 20.8 pg reticulocyte count - 4.2%). A peripheral smear was examined which showed microcytic hypochromic picture with numerous target cells, relative erythrocytosis and a Mentzer’s index of 8.4 strongly suggesting an underlying hemoglobinopathy (Figure 1).

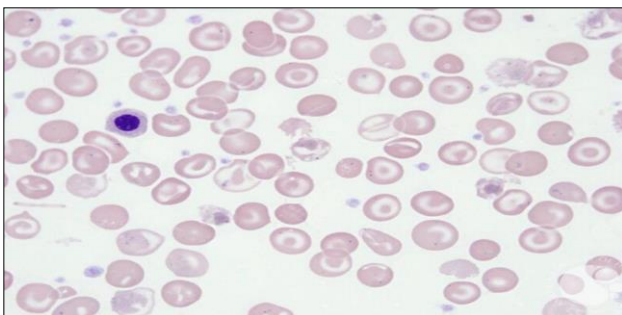


Figure 1: LG stain (40X) microphotograph depicting the classical thalassemia trait PBS features with relative erythrocytosis and microcytic hypochromic red cells. Numerous target cells and an erythroblast can be appreciated in the picture.

Table 1: Genetic, hematologic and disease related factors causing interference in HbA1C.⁴

Factors	
Erythropoiesis	Increased Hb A1C - iron and vit B12 deficiency
	Decreased Hb A1C - therapy with haematinics, erythropoietin, chronic liver disease
Altered Hb	Hemoglobinopathies/Hb F/meth hemoglobin
Glycation	Increased Hb A1C - alcoholism , kidney disease
	Decreased Hb A1C - aspirin, vitamin C/E therapy
Erythrocyte destruction	Increased Hb A1C - increase red cell life span , splenectomy
	Decreased Hb A1C - decreased red cell life span, splenomegaly drugs like antiretrovirals
Assay interference	Increased Hb A1C - increased bilirubin, variant Hb
	Decreased Hb A1C - variant Hb, increased triglycerides

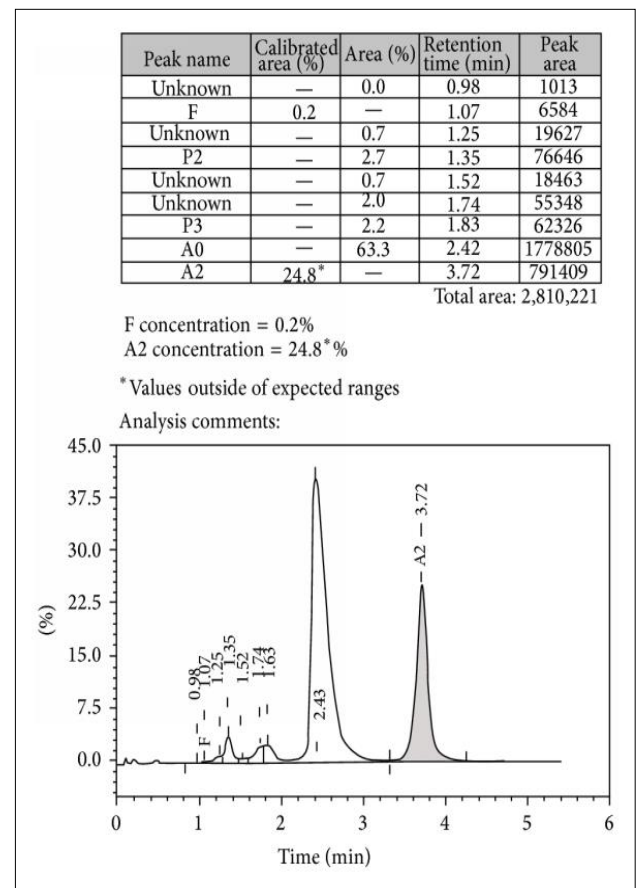


Figure 2: HPLC Chromatogram by Automated HPLC analyzer which revealed HbA0 of 63.3%, HbA2 24.8 (s/o presence of heterozygous Hb E) and Hb F of 0.2%.

Based on CBC and PBS findings a Hb HPLC was advised which revealed HbA0 of 63.3%, HbA2 24.8% and HbF of 0.2% (Figure 2). Thereby confirming the presence of asymptomatic Hb E in the patient.

The patient was counselled about the diagnosis and its implication. The clinician was informed regarding the alternate ways of measuring glycemic control in this patient. Further, his family screening revealed his son also to be carrier of Hb E disease for whom essential counselling was done.

DISCUSSION

Asymptomatic hemoglobinopathies are seen in almost 2-3% of Indian population. Though asymptomatic for the patients, these mild genetic defects carry important implications in patient's life especially at the time of marriage. The most common abnormal hemoglobin encountered in India are Hb S, Hb E, Hb D, Hb J meerut, Hb Q India.⁷ The HbE variant is extremely common in South east Asia and in north eastern part of Indian peninsula.⁸

Hb E basically contains a substitution of lysine for glutamic acid at position 26 of the β chain resulting in disorders varying from asymptomatic(heterozygous) to mild disease (homozygous). Subjects with heterozygous E trait are usually asymptomatic hence remain undiagnosed unless investigated specifically for hemoglobinopathies. Hb E interferes with HbA1C levels measured by ion exchange HPLC method, as the mutation tends to alter the ionic charges on the Hb thereby leading to co-elution of HB E along with HbA1C.⁹ The machine shows an abnormally high value of Hb A1C (in presence of Hb E) which is abnormally high or sometimes physiologically not possible. Hence, the clinicians should be aware of such interferents and should interpret the results accordingly. In such cases, the clinicians are advised to resort to subcutaneous monitoring of glucose or alternate methods of measuring Hb A1C which might not be affected by presence of abnormal Hb. Immunological methods or boronated affinity chromatography has been found useful in patients with hemoglobin variant.¹⁰ However, since these techniques are not available commonly, subcutaneous implants for glucose measurement are lucrative alternatives for checking of long-term glycemic control.

CONCLUSION

To conclude, it is important to be aware that there are many potential factors which interfere with HbA1C measurement, HB E being one of them. Inappropriate HbA1C measurements in persons with diabetes impacts their treatment and further management. Finally, a repeated flagged result must be pursued in detail to its

final conclusive cause which sometime may reveal an underlying pathology.

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