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Case Report

INTERFERENCE OF THE HEMOGLOBIN E WITH HEMOGLOBIN A1C ANALYSIS

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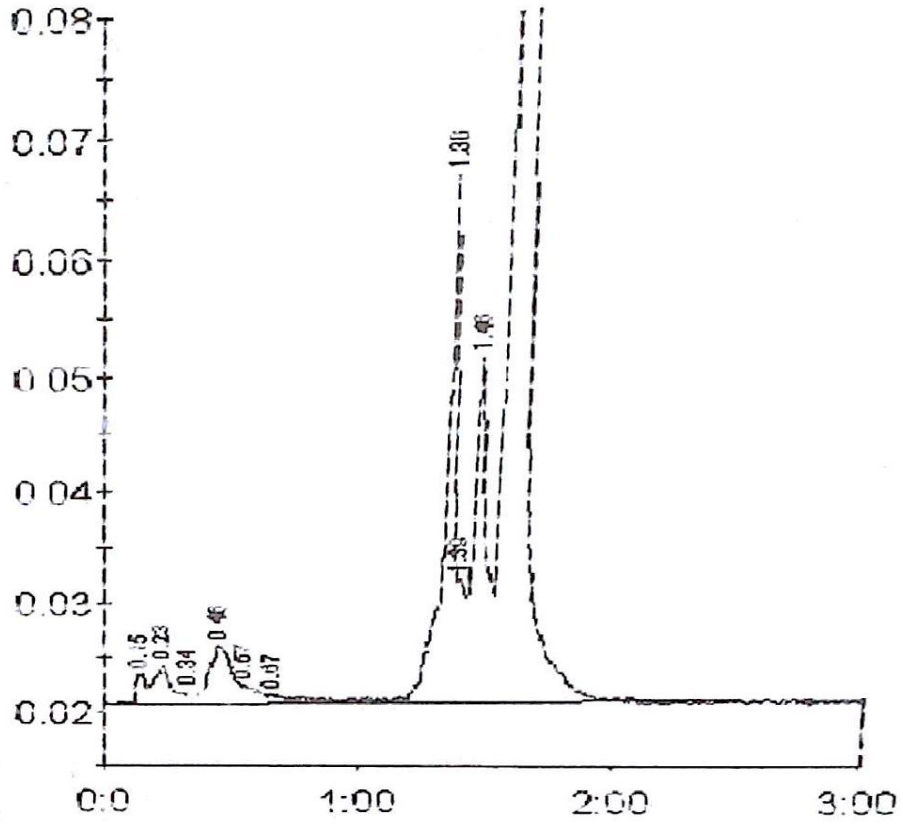
ABSTRACT
Hemoglobin A1c (HbA1c) is now considered to be the marker of choice in diagnosis and management of diabetes mellitus, based on the results of certain landmark clinical trials. Here in we presented a case of 48 years male patient came to biochemistry laboratory for checking of diabetic package. His HbA1C report on D10 HPLC was 0.0%. This case was evaluated for possible inference and Hemoglobin capillary electrophoresis was done which showed presence of HbE, HbS and elevated HbF fractions. Through this case study, we critically discuss the limitations of various HbA1c assay methods, highlighting the fact that laboratory professionals need to be aware of occurrences of Hb Hope, to help ensure patient safety

Keywords: *Diabetes Mellitus*; *Hemoglobin A1c (HbA1c)*

INTRODUCTION
Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease Glycated hemoglobin is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose 3. The measurement of glycosylated hemoglobin (GHb) is one of the well established means of monitoring glycemic control in patients with diabetes mellitus 4. The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was first proposed in 19765. Measurement of glycated hemoglobin is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels. According to the American Diabetes Association guidelines the glycosylated hemoglobin test can be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals 7. Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations [64] Management concentrates on keeping blood sugar levels as close to normal, without causing low blood sugar.

CASES
48 years male patient came to biochemistry laboratory for checking of diabetic package (FBS, PPBS, Hba1C, Microalbumin). The patient belonged to Assam State from north east part of India. His fasting and postmeal glucose values were 158 mg/dl (reference range < 100 mg/dl) and 282 mg/dl (reference range < 140 mg/dl) respectively. Microalbumin was 18 mg/ml (reference range 0 - 20 mg/ml). His HbA1c level, as measured by cation exchange high performance liquid chromatography (HPLC) via Bio-Rad D10 immunoassay (Bio-Rad Laboratories Inc, Hercules, CA), was 0.0%. Looking at the value it was suspected to be due to some type of interference. Considering the facts the same sample was subject to Hemoglobin electrophoresis using Sebia mini Cap capillary electrophoresis to find out the type of interfering hemoglobin. The Hb electrophoresis yield report as: HbS 6.2%, HbE 89.3% and HbA2 4.5%. The pattern is suggestive of Heterozygous HbE pattern.

Case Report

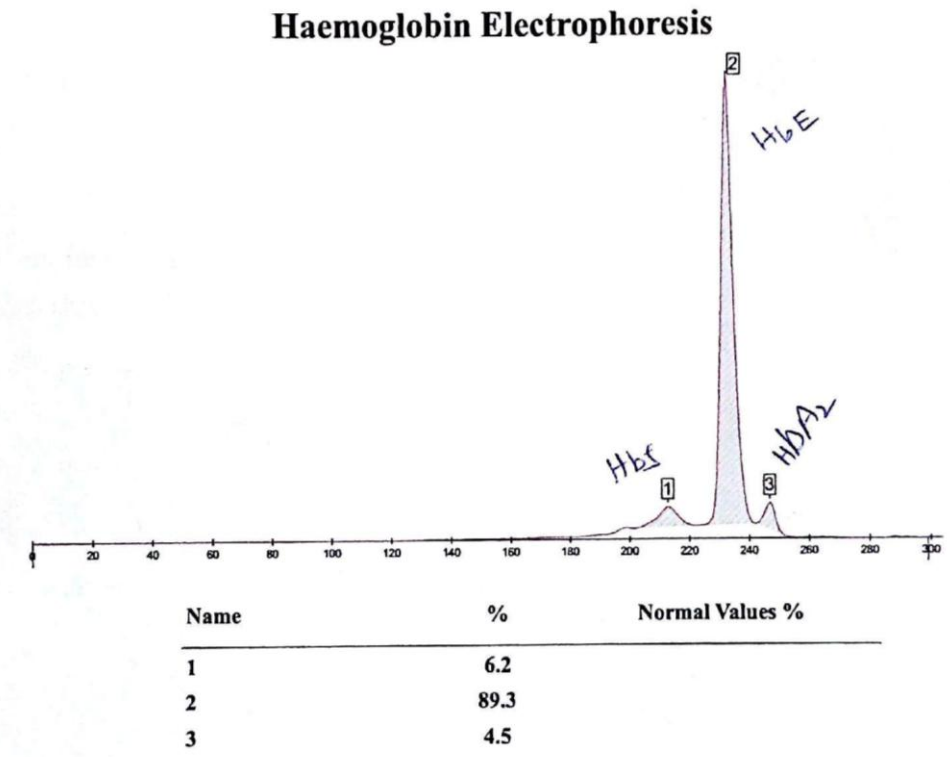


Peak table - ID: 2016275979

Peak	R.time	Height	Area	Area %
Unknown	0.15	2805	7744	0.4
A1a	0.23	3597	15415	0.9
A1b	0.34	1266	4663	0.3
F	0.46	5260	32864	1.8
Unknown	0.67	1610	4791	0.3
LA1c/CHb-1	0.67	699	2605	0.1
P3	1.36	45504	131204	7.2
Unknown	1.39	11096	26295	1.5
A0	1.46	30708	105976	5.8
Variant-Window	1.58	612132	1481814	81.7
Total Area:		1813371		

Concentration:	%
—	0.0

Graph 1: Chromatogram Showing HbA1C Value 0.0% and a Variant Window of 81.7% HbF 1.8%



Name	%	Normal Values %
1	6.2	
2	89.3	
3	4.5	

Graph 2: Electrophoretogram Showing HbE 89.3%, HbS 6.2% and HbA2 4.5%

DISCUSSION

Capillary electrophoresis testing for Hb has identified HbE. Hemoglobin E or haemoglobin E (HbE) is an abnormal hemoglobin with a single point mutation in the β chain. At position 26 there is a change in the amino acid, from glutamic acid to lysine. Hemoglobin E has been one of the less well known variants of normal hemoglobin. It is very common in Southeast Asia but has a low frequency amongst other ethnicities. Compound heterozygotes with hemoglobin sickle E disease result when the gene of hemoglobin E is inherited from one parent and the gene for hemoglobin S from the other. The βE mutation affects β-gene expression creating an alternate splicing site in the mRNA at codons 25-27 of the β-globin gene. Through this mechanism, there is a mild deficiency in normal β mRNA and production of small amounts of anomalous β mRNA. The reduced synthesis of β chain may cause β-thalassemia. Also, this hemoglobin variant has a weak union between α- and β-globin, causing instability when there is a high amount of oxidant (Chernoff *et al.*, 1956). Several laboratory methods are available for A1C measurement; boronate affinity or affinity-binding chromatography, cation-exchange chromatography, electrophoresis, and immunoassay are the most commonly used. Each laboratory method for A1C determination is based on the physical, chemical, or antibody-recognized properties of the normal (HbA) hemoglobin molecule.

Analytic interferences have been investigated for the majority of the common, commercially available HbA1c methods in the presence of heterozygous HbS, HbC, HbE, and HbD19–24 and are summarized at www.ngsp.org (accessed Dec, 2016). HbA1c can be measured in clinical laboratories by means of immunoturbidimetric assay (immunoassay), enzymatic assay, boronate affinity, ion-exchange HPLC, or capillary electrophoresis (CE). However, some methods and/or manufacturers' instructions alone do not provide sufficient information for making the correct decision about reporting analytically accurate or clinically useful results. It is important that each laboratory understands the analytic advantages and challenges associated not only with the general HbA1c method but also with the specific assay they

operate.

HPLC and immunoassay are the most commonly used methods to measure A1c. Immunoassays use antibodies that target the N-terminal glycated amino acids of the beta chain. Thus, any mutation in this epitope will produce spurious results via immunoassay

Testing of HbA1c levels via capillary electrophoresis is a relatively new but well-validated method that separates A1c and other Hb fractions via charge difference at high voltage using electro-osmotic flow. This method can be useful in patients who possess such variant hemoglobins because it has a longer runtime, leading to better resolution. Capillary zone electrophoresis is very precise and accurate for A1c estimation. It is not prone to common interferences and has the ability to detect several hemoglobin variants (Heylen *et al.,* 2014). Borbely *et al.,* (2013) have observed some uncommon variant hemoglobins that are detected by capillary electrophoresis but not HPLC, although the reverse also occurs in some cases. In chromatogram of HbA1C variant window is seen comprising area of 81.7%. Such chromatograms are essentially unacceptable. The interference HbE exerts on the cation exchanged "based methods could be due to several factors: HbE may affect the total hemoglobin determination. HbE may also co-elute with HbA1 and HbA1c fractions. HbE was reported earlier to affect the results of total hemoglobin by giving a falsely raised value (Normah and Kyi, 1988-1990). The co-elution with HbE on cation-exchange columns is another possibility. Other hemoglobin variants such as HbS and HbC had been reported to co-elute with the glycosylated hemoglobins resulting in the raised HbA1 or HbA1c levels.

Conclusions and Recommendations

This study shows that HbE affects the determination of HbA1 and HbA1c using kits as determined by cation exchange chromatography with low-pressure system.

HbE does not affect the determination of HbA1c using methods based on specific antibody to the glycosylated terminus of the 13-chain. Thus, it is recommended that of HbE positive individuals, the determination of their HbA1 (or HbA1c) should be carried out using capillary electrophoresis which is unaffected by HbE fraction. Also, it is advised to use alternate method for hyperglycemia detection like fructosamine or glycated albumin.

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