



## Variant hemoglobin (Hb hope) may enhance glycosylated hemoglobin (HbA1c) results of cation exchange high performance liquid chromatography

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### Abstract

*HbA1c estimation is routinely done in our lab by cation exchange high performance liquid chromatography (HPLC) method. During 2 year period, we estimated the EDTA venous blood samples of ~5000 patients. Surprisingly, in 2 patients abnormally raised HbA1c values were detected which were not in line with blood glucose levels (abnormal separation). One of the patients gave HbA1c value of 19.9% which suggested coelution of an hemoglobin variant along with glycosylated hemoglobin. Later, capillary electrophoresis (CE) of the sample showed an abnormal minor peak of a variant Hb adjacent to HbA; its concentration was 1.2%. Similarly, another patient also gave high A1c value of 21.2% by HPLC; CE electrophoregram of the sample showed a minor variant peak (1.4%) abutting HbA. Hb variant appeared to alter the HbA1c result and later required correction using another method.*

**Keywords:** Abnormal hemoglobins falsely interfere A1c results.

### Introduction

Management of diabetes mellitus (DM) depends on accurate measurement of HbA1c. The level of HbA1c is 4 to 6% in healthy subjects without diabetes. We had 2 patients with uncontrolled DM with abnormally high values of HbA1c (19.9% and 21.2%) by HPLC which appeared improbable. Later, CE electrophoregram of both EDTA blood samples showed minor peaks of an hemoglobin variant adjacent to major HbA peak. Herewith, we report 2 cases with heterozygous Hb variants which appeared to enhance the true value of HbA1c.

### Case Report

One of our patients was a female, aged 57 years. She had fasting blood sugar of 502 mg/dl. PP blood sugar after 2 hours of meals was 775 mg/dl. In addition, she had asymptomatic mild microcytic anemia (Hb 10.9 gm/dl) with raised RBC count (6.59 million/mm<sup>3</sup>). Processing of blood EDTA sample of this patient unexpectedly gave a raised value of HbA1c (19.9%) which suggested overlapping with a variant hemoglobin (table 1). Later, CE electrophoregram of the same sample was done which showed a minor peak of a variant Hbas Z10 zone adjacent to HbA (figure

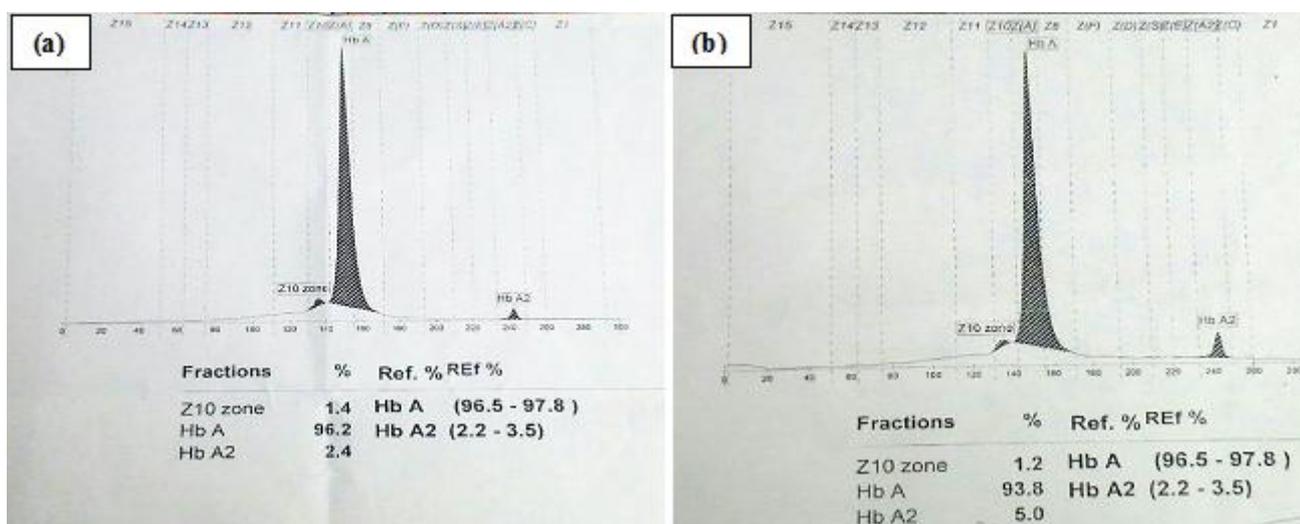
1a). Another patient was a male, aged 36 years. He had fasting blood sugar level of 320 mg/dl and PP blood sugar 2 hours after meals was 560 mg/dl. A1c result by HPLC method gave a high value of 21.2%; Hb variant value CE electro-

phoregram was 1.4% (table 1). Processing of samples from both the patients by HPLC method showed interference by an Hb variant (figure 1); both Hb variant and HbA1c eluted in the same window leading to overestimation.

**Table 1 :** HPLC and capillary electrophoresis findings of two patients

Lab ID	Age in years/ Gender	HbA1c result by HPLC (%)	Hemoglobin hope result by capillary electrophoresis (%)	Corrected A1c value
091803290020	57/F	19.9	1.2	18.7
100241	36/M	21.2	1.4	19.8

M = male, F = female



Graph showing a minor variant hemoglobin peak as Z10 zone on the capillary electrophoregram of (a) patient ID 091803290020 and (b) patient ID 100241.

**Discussion**

Electrophoresis depends upon charge differences on various hemoglobins. HPLC depends on the interchange of charged groups of the ion-exchange material with charge groups on the Hb molecule<sup>1</sup>. In current case report, HPLC gave falsely elevated HbA1c values which later required correction. Capillary electrophoresis at alkaline pH 9.4 gave separate minor peak of aHb variant adjoining to major HbA peak. Several hemoglobin variants, e.g. Hb hope, Hb Novakchoff, Hb Wayne, HbIwale (M-Kankakee), Hbcanden (Tochuchi) may interfere with HbA1c and give spuriously raised result by HPLC method. Results of current report suggest that the possibility of abnormal Hb interfering with HbA1c peak should always be considered if it is not in line with blood glucose levels. Initial very

high value of HbA1c by HPLC (>15%) should always be evaluated by another method. One of the hemoglobin variants (Hb hope) was first described about 100 years ago<sup>2</sup>. Although Hb hope is clinically silent, it can impair tissue oxygen delivery<sup>3</sup>. Abnormal Hb variant has been reported in some families of African, Japanese and Thai origin<sup>4</sup>. Per test results using Bio-Rad D10 HPLC analyser, Hb hope and other variants coelute with HbA1c giving spurious results<sup>5</sup>. Therefore, the possibility of interference by a silent Hb like Hb hope should always be discussed while analyzing the abnormally raised HbA1c value. Moreover, several methods for estimation of glycosylated Hb should be used while dealing with aberrant raised HbA1c value<sup>6</sup>. The laboratories should have alternative assay systems such as glycated albumin to assist the

management of the DM patient with a hemoglobin variant. One of our patients had mild anemia which suggested the finding as heterozygous Hb variant. Abnormal hemoglobin neither caused alterations in RBCs nor produced symptoms, suggesting possibility of hemoglobin hope<sup>2</sup> in our patients.

### Conclusion

Physicians heavily depend on the results of HbA1c while managing a case with Diabetes mellitus. Very high HbA1c value was obtained by HPLC chromatogram which may be improbable. Later, the results were corrected using another method. Moreover, alternative tests, e.g. glycated albumin should be available. Further, new methods are being developed such as mass spectrometry and another method which requires quenching of the fluorescence of an eosin-boronic acid solution.

**Financial and competing interests:** None

### Acknowledgement

We appreciate the support and help of Professor Dr. Sanjay Mehrotra.

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