Case Report

Uncontrolled Diabetic Subject With No HbA1c Value – A Case Report

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Abstract

A fasting blood specimen from a 33 years old diagnosed diabetic female subject was sent from one of our satellite centers to the department of Biochemistry, Bangladesh Institute of Health Sciences, Dhaka for hemoglobin A1c (HbA1c), fasting and 2 hours after breakfast (post-prandial) plasma glucose estimation. We found no value of HbA1c by HPLC method by repeated attempts. Chromatogram of HPLC showed no peak for HbA1c and a very low peak for HbA0 (the major hemoglobin). Data was presented as variant windows in the D-10TM hemoglobin assay system. Hb electrophoresis confirmed the Hb variant as HbE.

Key words: Glycosylated hemoglobin, Diabetes mellitus, Hemoglobin variant, Hemoglobin E, HPLC

Introduction

HbA1c is considered as a marker of long-term glycemic status of the previous two to three months in subjects with diabetes. In a recent study, HbA1c was translated to average glucose (eAG)[1] which can be used to get the approximate plasma glucose level. But the existence of some hemoglobin variants limits the use of the equation. Presence of some hemoglobin variants is one of the causes of deterioration of HbA1c values. Approximately 1,000 variants have been defined[2], of them HbS, HbC, HbE and HbD are common in different populations. Except a few, most of the Hb variants are silent and possess no clinical significance. But the influence of Hb variants on HbA1c measurement is remarkable[3,4,5]. Moreover, the prevalence of HbE in the south-east Asia is higher[6] and in most cases it is silent. Measurement of HbA1c can be done by immunological, enzymatic and cation-exchange high performance liquid chromatographic methods. Recently two cases of diabetic subjects were described in a study carried out in Japan, one of the subjects was a citizen of Myanmar and the other was a citizen of Thailand, with immeasurable HbA1c values by cation exchange high performance liquid chromatography (HPLC) (HA-8160, Arkray, Tokyo). Electrophoresis and isoelectric focusing confirmed the presence of hemoglobin variant HbE[7]. In our daily practice, we deal with a large number of HbA1c specimens and we observed some deteriorated HbA1c values. The study subject was one of them.

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Case Report

The age of the female study subject was 39 years. Fasting and post-prandial plasma glucose levels were 12.4 mmol/L and 12.1 mmol/L. She had a 3 years history of diabetes mellitus (DM). She has no family history of DM. Her body mass index was 24.1 Kg/m2 and her blood pressure was 130/80 mmHg. The study subject continued her medication (oral hypoglyceamic agent) to control blood glucose level, but did not achieve the target of therapy. She had no habit to take alcohol. She was pregnant (3 months). Serum creatinine concentration was normal but serum ALT was slightly elevated. The HbA1c value (measured by D-10TM, hemoglobin assay system, Bio-Rad, USA) was 0.0% in two repeated measurement in two different occasions at two days interval. The chromatogram of the HPLC showed no peak for HbA1c (0.0%) and a very low peak for major hemoglobin HbA0 (4.7%) whereas an undesirably high peak for Hb variant (79.8%) (figure 1). Written informed consent was obtained from the subject prior to further investigation and to conduct the study. Hemoglobin electrophoresis (Hydragel) confirmed the

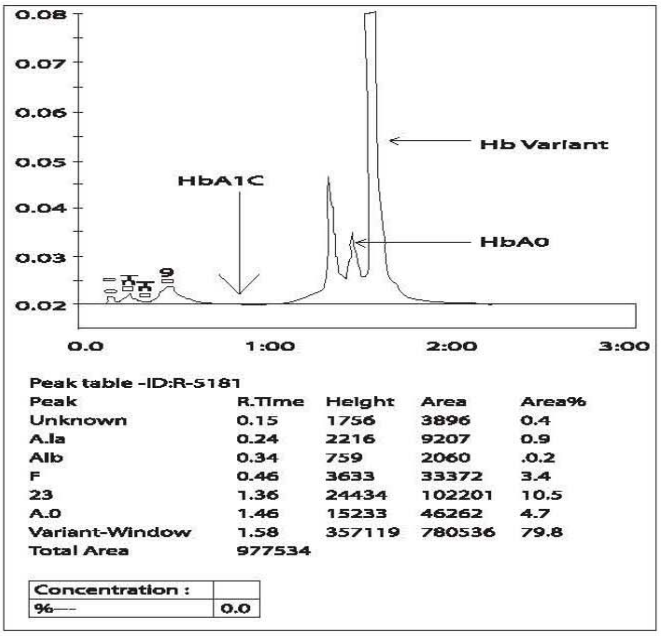

Figure 1: Chromatogram of HPLC

variant as HbE (figure 2). HbA1c measured by Variant (Bio-Rad, USA) hemoglobin assay system that measured all the variants, yielded HbA1c value of 8.2%. The HbA1c value measured by immunoassay was 8.0 %. Her blood

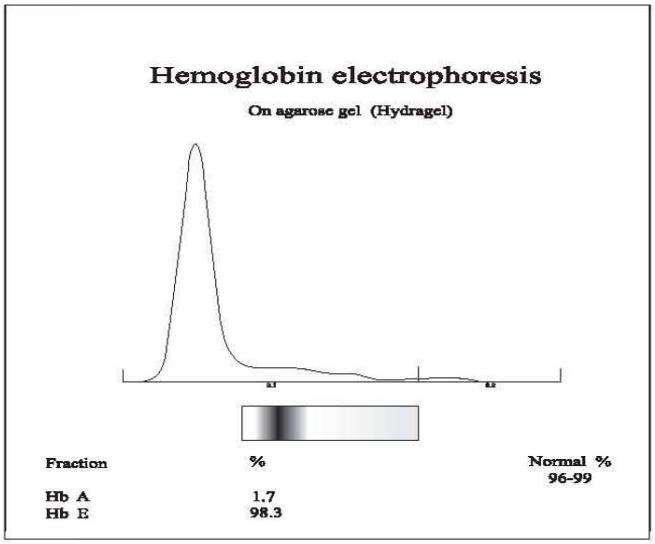

Figure 2: Hemoglobin electrophoresis

examination showed a low Hb, RBC and MCV level, total cholesterol was slightly elevated with normal TG. Her HDL cholesterol was 40 mg/dL and LDL cholesterol was 152 mg/dL. Laboratory data of the subject is shown in table 1.

Table 1: Laboratory data

CBC	
Hb	9.5 g/dL
HCT/PCV	0.31 L/L
MCV	55.7 fL
MCH	17.2 pg
MCHC	30.9 g/dL
WBC	13.8×10^9/L
RBC	3.6×10^{12}/L
Platelet	176×10^9/L
Blood Chemistry	
S Total Protein	77.9 g/L
S Albumin	40.4 g/L
T Bil	0.4 mg/dL
ALT	53 U/L
AST	23 U/L
ALP	117 U/L
CK	56 U/L
S Creatinine	0.9 mg/dL
S Na^+	137 mmol/L
S K^+	3.7 mmol/L
Total Cholesterol	218 mg/dL
TG	131 mg/dL
HDL cholesterol	40 mg/dL
LDL cholesterol	152 mg/dL
Fe	9 µmol/L
TIBC	41 µmol/L
Ferritin	53.1 ng/mL
Fasting plasma glucose	12.4 mmol/L
Post-prandial plasma glucose	12.1 mmol/L
HbA_{1c}*	0.0 %
HbA_{1c}**	8.2 %
HbA_{1c}***	8.0 %

*, Measured by D-10™ (Bio-Rad); **, Measured by Variant™ (Bio-Rad); ***,Measured by immunoassay.

Discussion

The case subject had Hb variant of HbE. Since D-10TM HPLC method cannot detect glycosylated hemoglobin variants, the value of HbA1c was not detectable by this analyzer. HbA1c is an established marker of glycemic status. Remarkable deterioration of HbA1c values is associated in subjects with hemoglobin variants. Falsely low or high values may be generated by different methodology. Use of serum glycosylated albumin or Glycosylated Perum Protein (GSP) was also suggested. Due to the high prevalence of HbE silent variants in our population, HbA1c values measured by HPLC method may be frequently deteriorated, which may misguide the treatment strategy of diabetic subjects. The D-10TM HPLC hemoglobin assay system is unable to detect HbE1c in the case of HbE. The result obtained by Variant HPLC was inconsistent with the result obtained by other HPLC based HbA1c analyzer[7] but correlated to plasma glucose level and also mimics to HbA1c value measured by an immunoassay. HPLC based hemoglobin assay system that can differentiate Hb variants from HbA is not suitable for HbA1c estimation when Hb variant is present. Hemoglobin variants may be easily identified by laboratory personnel from HPLC chromatogram. Alternative method should be sought in such cases to monitor glycemic status. Since glycosylation of HbA1 occurs at the N-terminal valine residue of the β chain and the mutation of the β globin chain of HbE occurs at codon 26 (GAG to AAG), HbE1c can be measured by immunoassay. Immunological methods are not recommended when mutation occurs up to position 10 of the β-globin chain. Enzymatic and boronate affinity chromatographic method can be used to measure glycosylated hemoglobin in subjects with HbE. Frequent blood glucose monitoring (self-monitor, cross-checked with standard method) may be helpful in this type of subjects in context of our country.

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