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CASE REPORT

False hemoglobin A_{1c} value as a result of compound heterozygotes of hemoglobin E and hemoglobin New York

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ABSTRACT

The presence of hemoglobin (Hb) variants might interfere with some glycated hemoglobin (HbA_{1c}) measurements. There have been a few reports of compound Hb variants affecting HbA_{1c} testing. Here, we report a case of the coinheritance of two Hb variants in the β-globin gene. High-performance liquid chromatography with the Hb program showed a high HbA_2 level. Similarly, an E-window peak was separated on the high-performance liquid chromatography with a glycated Hb program. However, capillary electrophoresis showed two abnormal peaks and no HbA peak. Sanger sequencing confirmed the presence of Hb New York and HbE. This is the first report of a compound heterozygote for HbE and Hb New York. The double heterozygote caused erroneous results for HbA_{1c} on high-performance liquid chromatography and enzyme assay.

INTRODUCTION

Glycated hemoglobin (HbA_{1c}) is widely considered the most reliable marker for diagnosing diabetes and monitoring glucose control[1]. There are many reports that the presence of a hemoglobin (Hb) variant might interfere with the measurement of HbA_{1c} results[2–4]. Heterozygous HbE, the common β-globin variant, possesses an influence on the HbA_{1c} value[5,6]. The effect of heterozygous Hb New York on HbA_{1c} value is observed in different assays[7]. However, little information is available on the determination of HbA_{1c} affected by compound Hb variants in the β-globin gene. Here, we report that the judgment of HbA_{1c} might be affected by Hb New York and HbE using high-performance liquid chromatography (HPLC) and enzyme assay.

CASE REPORT

A 26-year-old woman in the first trimester of pregnancy presented to the Women and Children's Care Hospital of Xing'an County, Guilin, Guangxi, China, for a pregnancy examination. Written informed consent was obtained from the patient, and samples were collected. The hematological parameters showed the following: Hb level 11.8 g/dL, red cell count 4.76×10^{12}/L, mean corpuscular volume 72.7 fL and mean corpuscular Hb 24.8 pg (Sysmex XN 550; Sysmex Corporation, Kobe, Japan). Her fasting blood glucose level was 5.0 mmol/L (AU680; Beckman Coulter, Brea, CA, USA). HbA_{1c} measurement was carried out using HPLC (D100; Bio-Rad, Hercules, CA, USA) and enzyme assay (AU680; Beckman Coulter).

According to local policy, a pregnant woman can receive free screening tests. Thalassemia screening was carried out using HPLC (VARIANT II™; Bio-Rad). Capillary electrophoresis (CE; CapillaryS2 Flex Piercing; Sebia, Lisses, Paris, France) was used to screen the potential Hb variants compared with HPLC. Routine genetic analysis was used to rule out the presence of α-thalassemia (Yishengtang Biotech, Shenzhen, China). Finally, Sanger sequencing was carried out with a 3,500 XL sequencer (Applied Biosystems, Foster City, CA, USA).

D100 showed HbF 1.24%, HbA_{1c} 5.15%, HbA0 54.89% and E-window peak 31.31%, which suggested the presence of HbE (Figure 1a). In view of the aberrant peak, the HbA_{1c} value was then determined by enzyme assay, and the HbA_{1c} value was 3.88%. Hb analysis showed a high HbA_2 value (28.9%) by VARIANT II, suggesting a potential Hb variant co-eluting with HbA_2 (Figure 1b). No mutations were detected by routine genetic analysis. Sanger sequencing detected a single-nucleotide substitution (G>A) at codon 26 in the *HBB* gene,

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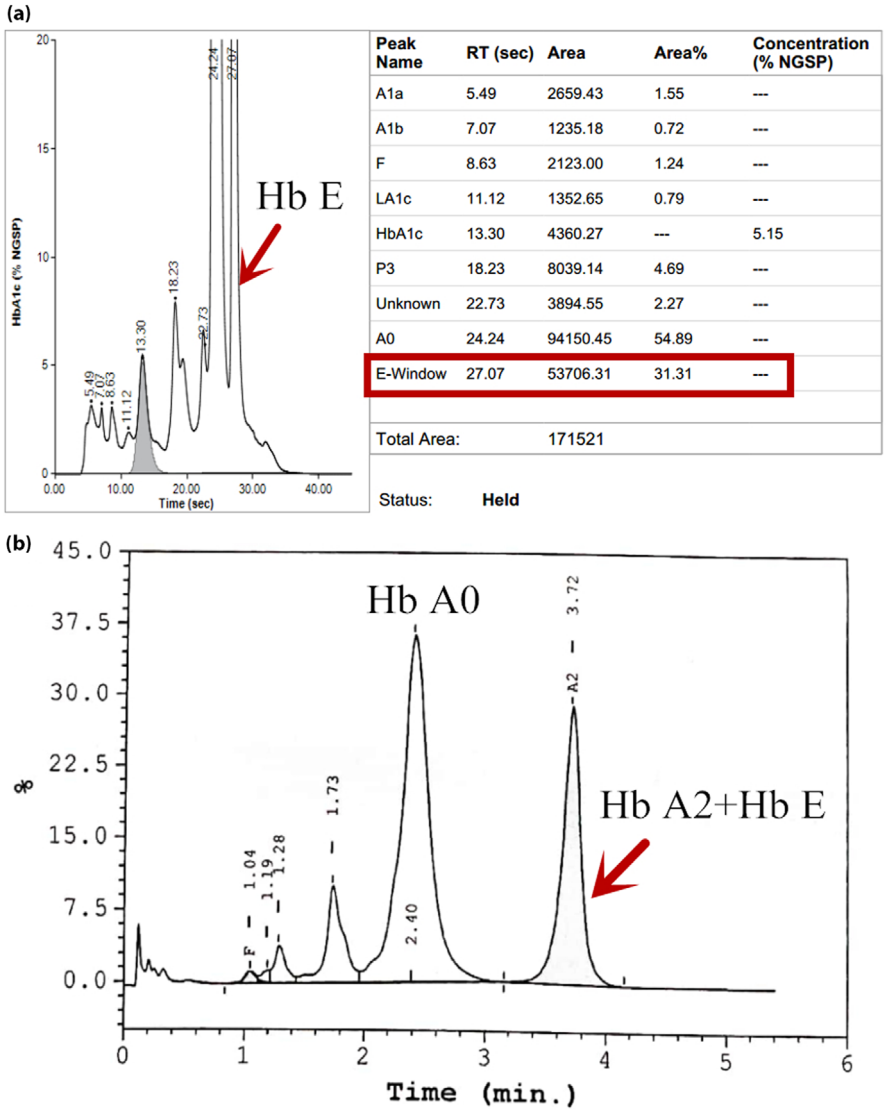

Peak Name	RT (sec)	Area	Area%	Concentration (% NGSP)
A1a	5.49	2659.43	1.55	---
A1b	7.07	1235.18	0.72	---
F	8.63	2123.00	1.24	---
LA1c	11.12	1352.65	0.79	---
HbA1c	13.30	4360.27	---	5.15
P3	18.23	8039.14	4.69	---
Unknown	22.73	3894.55	2.27	---
A0	24.24	94150.45	54.89	---
E-Window	27.07	53706.31	31.31	---
Total Area:		171521		

Status: **Held**

Figure 1 | (a) Glycated hemoglobin (HbA_{1c}) and (b) hemoglobin (Hb) analysis for the patient with compound heterozygous HbE and Hb New York by high-performance liquid chromatography.

corresponding to HbE (β26 [B8] Glu >Lys, *HBB*:c. 79 G>A; Figure 2a). Accidentally, we found that there was another mutation (T>A) at codon 113 in the *HBB* gene, which corresponded to Hb New York (β113 [G15] Val >Glu, *HBB*:c. 341 T>A; Figure 2b). Subsequently, CE was confirmed, and presented values of HbE 26.9%, Hb New York 71.3% and HbA_2 1.8% (Figure 3a).

To investigate more information, we carried out a pedigree study. Unfortunately, the patient had no brothers or sisters, and her father died. Her mother's (age 51 years) related measurement results were as follows: Hb 119 g/dL, mean corpuscular volume 80.6 fL, mean corpuscular Hb 26.2 pg; HbA 70.2%, HbA_2 3.6%, HbE 26.2% (by CE; Figure 3b); HbA_{1c} 5.4% by HPLC and HbA_{1c} 5.2% by enzyme assay; CD26 heterozygous mutation in the β-globin gene (Figure 2c).

DISCUSSION

In the present study, we detected compound heterozygous HbE and Hb New York in a patient with a false HbA_{1c} value, as measured by HPLC and enzyme assay. Interestingly, HPLC could not detect Hb New York in either glycation analysis or Hb analysis mode (Figure 1)[8]. If a CE re-examination was not carried out, the patient was likely to be considered heterozygous for HbE. The HbA_{1c} value by enzyme assay was 3.88%, whereas the HPLC was 5.15%. Based on the normal fasting blood glucose of the patient, the HbA_{1c} value by HPLC was

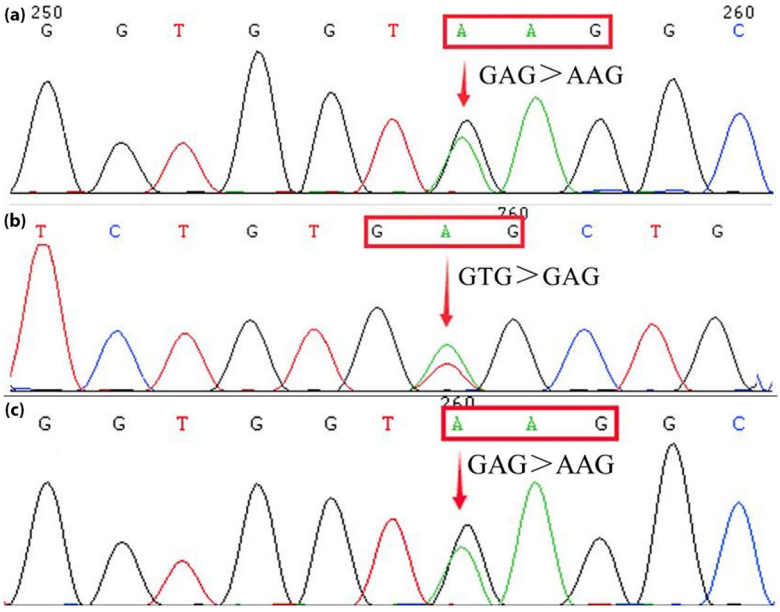

Figure 2 | Sanger sequencing showed compound heterozygous (a) hemoglobin (Hb)E and (b) Hb New York in the patient, and (c) heterozygous HbE in her mother.

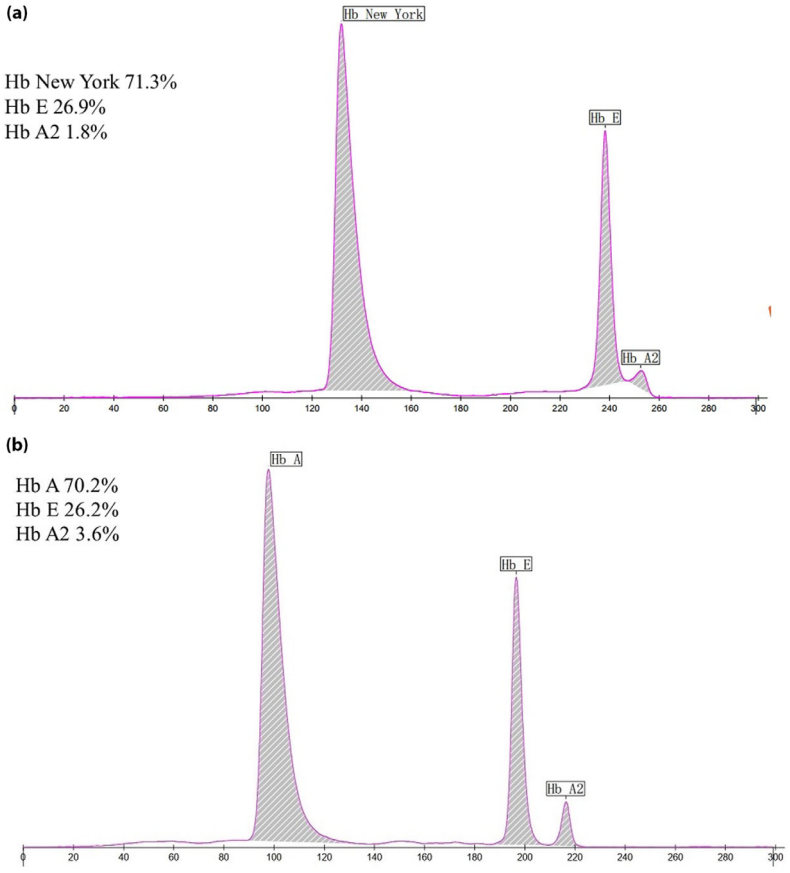

Figure 3 | Results of hemoglobin (Hb) analysis by capillary electrophoresis for (a) the patient and (b) her mother.

considered reasonable. However, Sanger sequencing showed two variant mutations on the β-globin chain, which suggested that the measurement HbA_{1c} results by HPLC and enzyme assay were incorrect.

As far as we know, enzyme assay is often considered a reference method when the Hb variants are presented. The patient was compound heterozygous HbE and Hb New York in the present study. It suggested no normal β-chain generation, and HbA could not be synthesized. The CE confirmed this speculation, and no HbA was detected. However, the present study showed a low HbA_{1c} value (3.88%) by enzyme assay. Obviously, this was a false HbA_{1c} value. Therefore, assays, such as HPLC and CE, are recommended to screen Hb variants or thalassemia, and avoid misdiagnosis of diabetes. Enzyme assay might be considered when the presentence of the Hb variant interferes with HbA_{1c} measurement. It was observed that the result of HbA_{1c} using HPLC was different from using the enzyme assay from the patient's mother. When double heterozygotes or homozygotes for β-globin chain variants are encountered, HbA_{1c} cannot be used as a detection indicator, regardless of the methods used.

In conclusion, this is the first report of the compound heterozygous HbE and Hb New York with false HbA_{1c} measurement. It should prompt clinicians to be careful about Hb variants when the HbA_{1c} value is outside the laboratory's reference range and not consistent with the clinical picture. Because Hb variants can lead to abnormal HbA_{1c} values through various mechanisms, no single method can accurately measure HbA_{1c}. As in the present study, both HPLC and enzyme assay received false HbA_{1c} results from the patient with compound Hb variants. Therefore, the assessment of glycemic control should be changed to use other indicators.

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: N/A.
Informed consent: Informed consent was obtained from participants in this study.
Registry and registration no. of the study/trial: N/A.
Animal studies: N/A.

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