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ORIGINAL ARTICLE

Identification of a new hemoglobin variant Hb Liuzhou [*HBA1*:C.182A→G] by MALDI-TOF mass spectrometry during HbA_{1c} measurement

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ABSTRACT

Structural hemoglobin (Hb) variant is generally caused by a point mutation in the globin gene that produces one amino acid substitution. Here, we describe a new α-chain variant found during HbA_{1c} measurement. HbA_{1c} procedures based on both ion-exchange high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) techniques failed to identify its presence. In contrast, MALDI-TOF mass spectrometry (MS) revealed the existence of a variant α-chain with a mass of 15155 Da. In addition, the Hb variant interfered with HbA_{1c} determined by Bio-Rad D100. DNA sequencing confirmed the occurrence of a new heterozygous mutation [*HBA1*:C.182A→G] at codon 60, resulting in a coding change from lysine to arginine. We named it Hb Liuzhou for thde birthplace of the patient. This case exemplified that MALDI-TOF mass spectrometry can serve as the method of choice to identify and quantify the Hb variant.

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Introduction

Hemoglobin (Hb) is the polypeptide tetramer consisting of two pairs of α and β globin chains. In healthy adults, over 95% Hb is HbA ($\alpha_2\beta_2$), with the remaining Hb of two minor components including less than 2.0% HbF ($\alpha_2\gamma_2$) and approximately 2.5–3.5% HbA_2 ($\alpha_2\delta_2$). Hemoglobin variant, characterized by structural defects in Hb, is one of the most common inherited monogenic diseases. More than 1350 Hb variants, arising mainly from α- or β-globin gene mutation, have been documented to date, and each one presents specific biological characteristics [1]. Although most Hb variants heterozygotes are asymptomatic, some compound heterozygotes or homozygotes produce significant clinical symptoms [2]. Therefore, identification of the Hb variant may be of great significance in genetic counseling and prenatal diagnosis.

Currently, the most popular methods to identify Hb components, as well as their glycated forms such as hemoglobin A_{1c} (HbA_{1c}), are based on cation-exchange high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) techniques [3,4]. Previously, Hb variants are occasionally recognized during routine HbA_{1c} testing [5], which received considerable attention because of the interference of Hb variants on HbA_{1c} results [6,7]. In addition to cation-exchange HPLC and CE, mass spectrometry (MS) has been used to analyze Hb variants [8]. In the present study, we report a new Hb variant found while quantifying HbA_{1c} by MALDI-TOF MS instead of the traditional separation methods such as cation-exchange HPLC and CE.

Materials and methods

Case description

A 23-year-old woman from Liuzhou of Guangxi province visited our hospital for a routine checkup. Her glucose

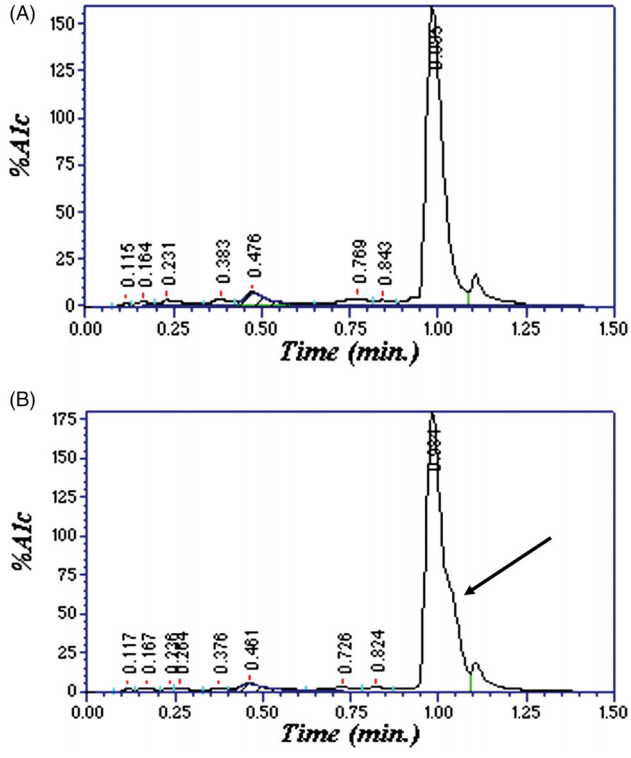

Figure 1. Expanding HbA_{1c} chromatograms from Variant II Turbo 2.0. (A) A normal expanding HbA_{1c} chromatogram. (B) Arrow indicates a convex curve on the right side of the chromatogram of the sample with Hb Liuzhou.

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result was 3.99 mmol/L (reference interval: 3.90–6.10 mmol/L). HbA_{1c} analysis was initially performed by Variant II Turbo 2.0 (Bio-Rad, USA) and an abnormal convex on the right side of expanding chromatogram was observed when compared with normal control (Figure 1). Therefore, we further tested the residual sample and discovered a new Hb variant. We named it Hb Liuzhou for the birthplace of the patient. This work was approved by the Ethics Committee of Peking University Shenzhen Hospital and informed consent was obtained from the woman.

HbA_{1c} and hemoglobin analysis

HbA_{1c} was reanalyzed using an HPLC system (D100, Bio-Rad, USA), a boronate affinity chromatography system (Ultra^2, Trinity Biotech, Ireland), a CE system (HbA_{1c} procedure; Capillarys 3 TERA, Sebia, France), and a MALDI-TOF MS system (QuanTOF, Intelligene Biosystems, China). All experimental procedures were carried out following the manufacturers' instructions. As a supplement when Hb variants are suspected in our laboratory, QuanTOF was conducted following the approach described in our early study [9]. Furthermore, Hb analysis was performed using the Hb procedure of CE on Capillarys 3 TERA.

DNA analysis

To confirm the occurrence of gene mutation, *HBA1*, *HBA2*, and *HBB* genes were sequenced using an ABI PRISM TM 3730 XL Sequencer (Applied Biosystems, USA). Before DNA sequencing, genomic DNA was extracted and then amplified with an S1000^{TM} Thermal Cycler PCR system (Bio-Rad). Amplification conditions and primers adopted were the same as those in our previous report [10]. Amino acids and codons of globin chains were described using the traditional nomenclature.

Hematological parameters analysis

To determine the patients' hematological characteristics associated with the Hb variant, the hematological data were measured with a hematology analyzer (Sysmex XN9000, Sysmex, Japan).

Results

HbA_{1c} and hemoglobin analysis

The HbA_{1c} results were 4.8% (29 mmol/mol, Variant II Turbo 2.0), 4.7% (28 mmol/mol, Ultra^2), 4.2% (22 mmol/

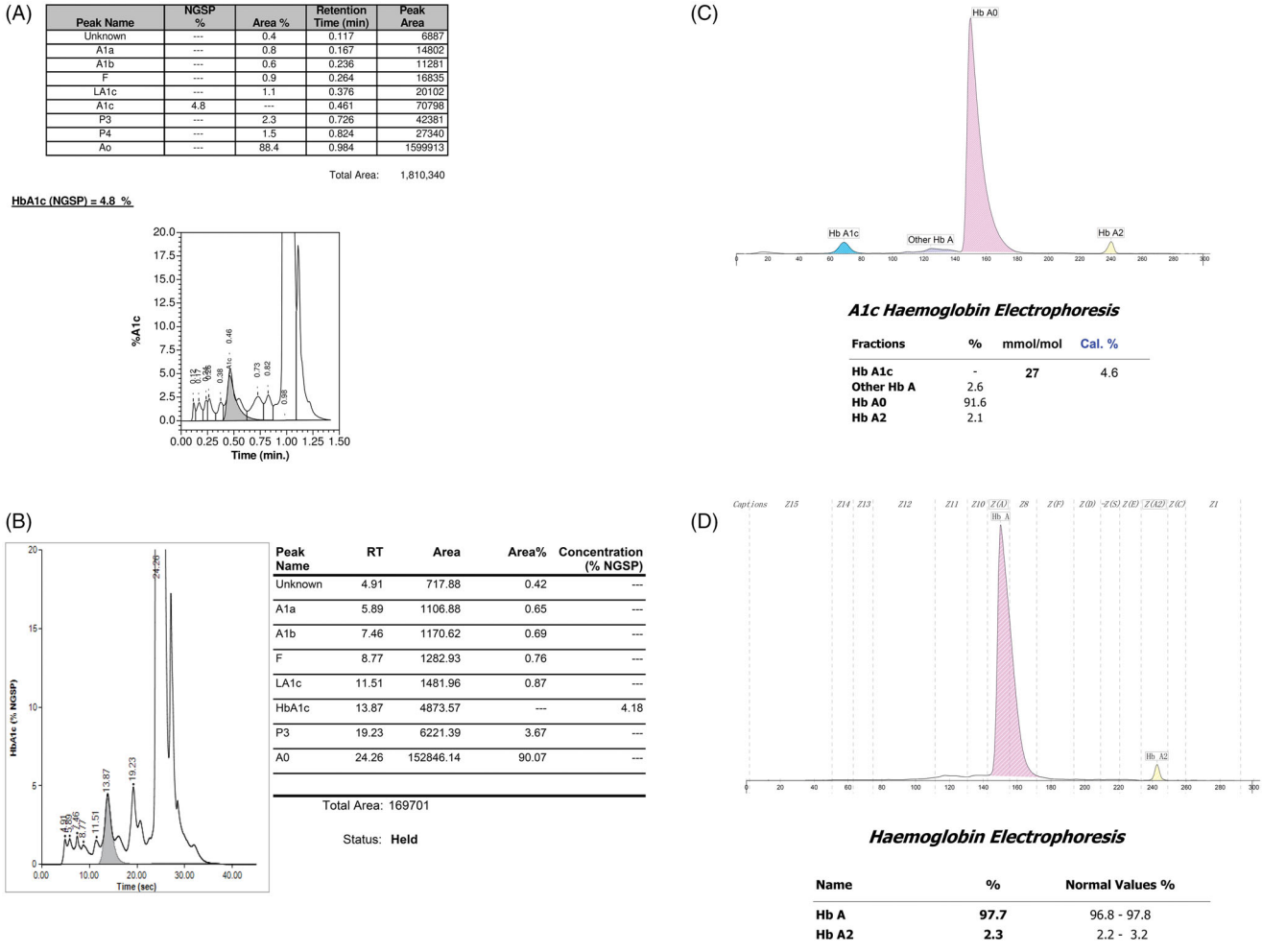

Figure 2. HbA_{1c} and hemoglobin analysis. HbA_{1c} measured by Variant II Turbo 2.0 (A), D100 (B), and Capillarys3 TERA (HbA_{1c} procedure) (C). Hemoglobin analysis by Hb procedure on Capillarys3 TERA (D).

mol, D100) and 4.6% (27 mmol/mol, Capillarys 3 TERA). No abnormal peaks were detected by any of the previously described HbA_{1c} techniques. Hemoglobin analysis also showed nothing abnormal with 97.7% HbA and 2.3% HbA_2 (Figure 2). Using Ultra[2] as a comparative method, as in early research [11], the relative biases in National Glycohemoglobin Standardization Program (NGSP) units were –2.1% (Variant II Turbo 2.0), –10.6% (D100), and 4.2% (Capillarys 3 TERA).

MALDI-TOF MS

QuanTOF yielded an HbA_{1c} value of 4.8% (29 mmol/mol). Meanwhile, a variant chain with a mass of 15,155 Da was found in the mass spectrogram, and the variant chain accounted for 26.4% of the total α-chain (Figure 3).

DNA analysis

No mutation was detected in *HBA2* and *HBB* genes. In contrast, Sanger sequencing identified a new heterozygous mutation [*HBA1*:C.182A→G] on *HBA1* gene (Figure 4), resulting in a coding change at codon 60 from lysine (molecular weight: 146 Da) to arginine (molecular weight: 174 Da).

Hematological parameters

Hermatological parameters were as follows: red cell count (RBC), 4.03 × 1012 (reference: 3.8–5.1 × 1012/L); Hb, 128 g/L

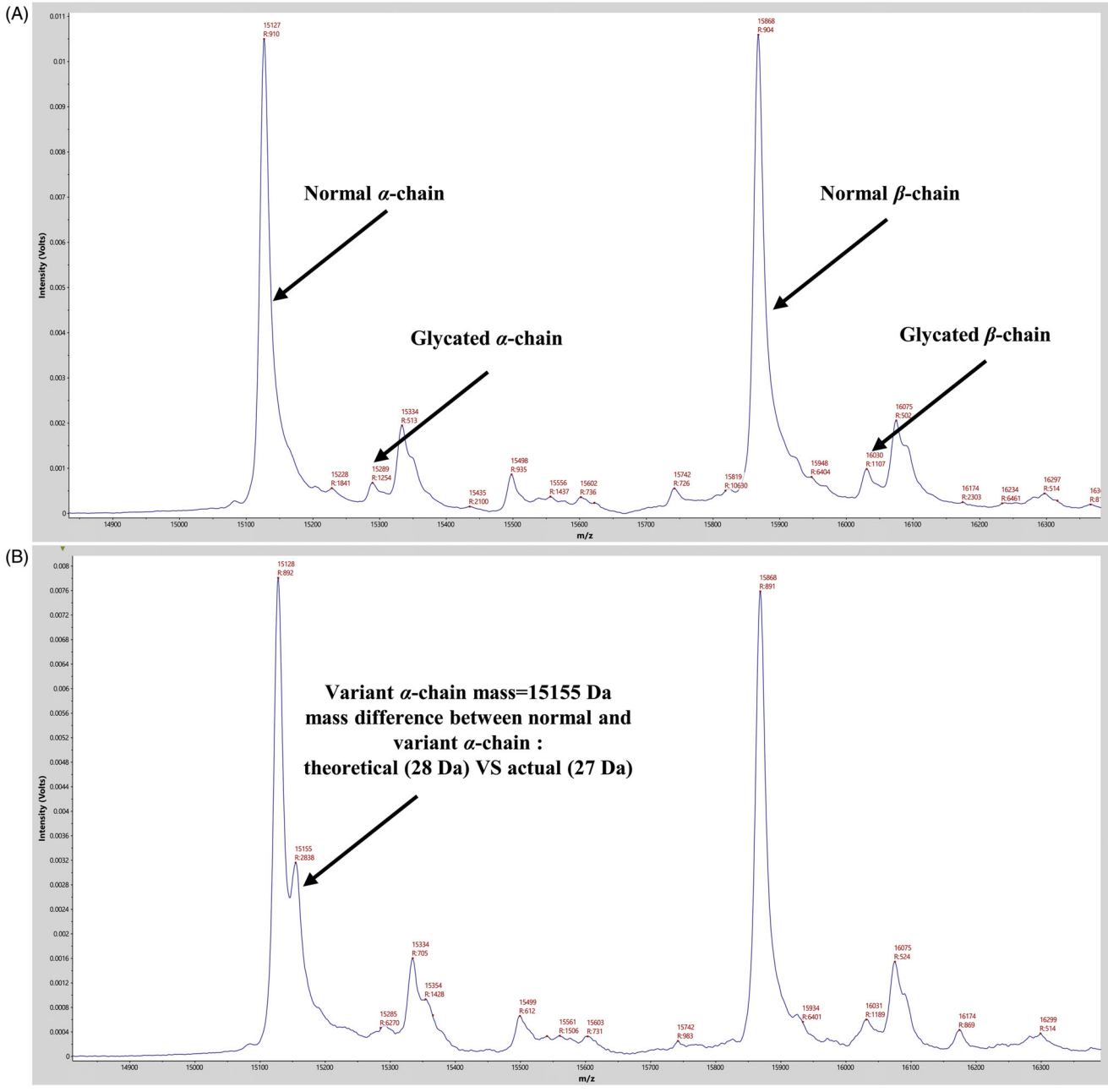

Figure 3. MALDI-TOF MS hemoglobin analysis. (A) Mass spectrogram of a non-variant sample showed α-chain (15,127 Da), β-chain (15,868 Da), and the corresponding glycated α-chain (15,289 Da) and glycated β-chain (16,030 Da). (B) Mass spectrogram of Hb Liuzhou showed a variant α-chain peak (15,155 Da).

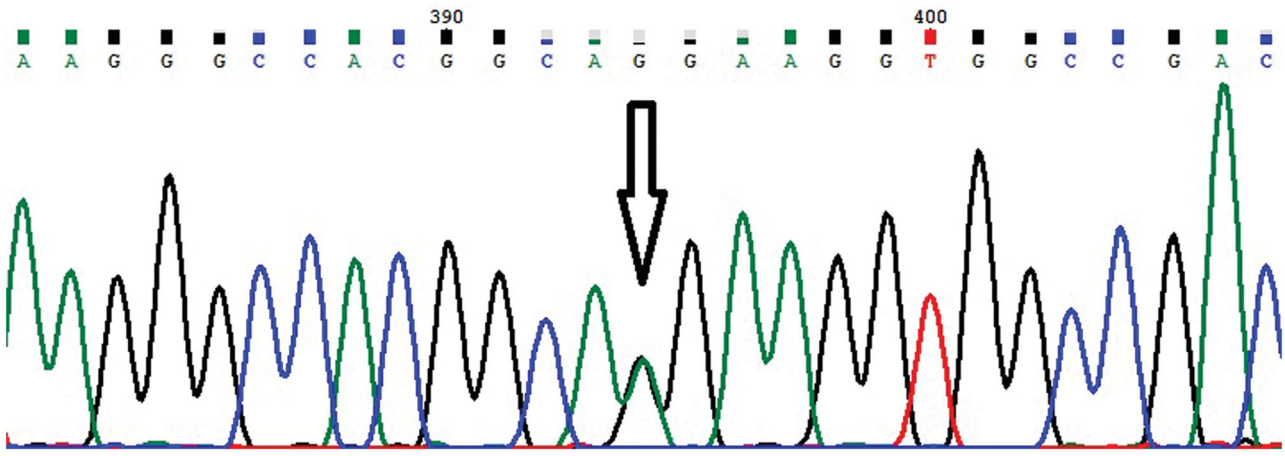

Figure 4. Sanger sequencing results of Hb Liuzhou. Arrow indicates a heterozygous mutation [*HBA1*:C.182A→G] in *HBA1* gene.

(reference:115–150 g/L); mean corpuscular volume (MCV), 99.0 fL (reference: 82.0–100.0 fL); mean corpuscular Hb (MCH), 31.8 pg (reference: 27–34 pg). The normal RBC parameters indicated the presence of a hematologically silent variant.

Discussion

HbA_{1c}, a universally accepted biomarker for diabetes management, is defined as the attachment of glucose to the N-terminal valine residue of β-chain, resulting in a mass increase of 162 Da originated from the elimination of water (18 Da) from a glucose adduct (180 Da). Currently, a large number of commercial instruments, based mainly on immunoassay or separation techniques such as cation-exchange HPLC and CE, are available for the determination of HbA_{1c}. Besides, even though MALDI-TOF MS is still not being used as a routine method for HbA_{1c} measurement due to some limitations such as high-cost equipment, complex manual operation, and high requirements for personnel expertise, it had been employed to estimate the quantity of globin chain and glycated forms years ago [12,13]. A significant difference of using MALDI-TOF MS compared to conventional HbA_{1c} methods is that MALDI-TOF MS analysis allows the discrimination of single globin chains but not Hb tetramers consisting of four globin chains because the tetramer was denatured before its injection in the MS system.

Although improved assay performance of commercial HbA_{1c} analyzers has eliminated many interference of Hb variants on HbA_{1c} analysis, many Hb variants still hamper HbA_{1c} quantification [7,8]. According to the latest NGSP criterion, an acceptable bias for HbA_{1c} was defined as within ± 5.0% (relative difference). In the present study, unacceptable bias was only found for D100 when compared with Ultra[2] result. In addition to the boronate affinity HPLC method, an immunoassay can also be used as a comparative method for HbA_{1c} measurement [6]. On account of the lack of HbA_{1c} value obtained with immunoassay, the very meaningful comparison of separation or mass discrimination techniques with an immunoassay cannot be achieved.

As MALDI-TOF MS cannot distinguish between glycation sites with the same mass, the resulting signal for glycated chains represents total single glycated Hb [12,13]. Despite this limitation, in this case, HbA_{1c} values from total single glycation correlated well with the comparative method. Additionally, HbA_{1c} determined through the β-chain glycation of MALDI-TOF MS, may not be prone to the interference of variant α-chain because of the large mass difference between α-chain (15,127 Da) and β-chain (15,868 Da).

MALDI-TOF MS has been the tool of choice for characterizing Hb variants by the use of their mass differences. Previous studies demonstrated MALDI-TOF MS enabled mass discrimination of approximately 15–20 Da [14]. Unlike MS, the separation power of cation-exchange HPLC and CE techniques mainly rely on the charge state of the Hb variant. The majority of the frequently detected variants are charge-difference substitutions; otherwise, it will not be detectable and result in the overlap of Hb variants and HbA peaks. As seen in this study, cation-exchange HPLC and CE methods faced challenges in detecting the new Hb variant. However, MALDI-TOF MS detected the variant chain *via* the sufficient mass difference between the normal and variant chain. Moreover, the theoretical variation of molecular weight (28 Da) matched the measured mass difference of 27 Da. In general, the percentage of Hb variants due to a *HBA2* gene mutation is higher than that due to an *HBA1* gene mutation [15]. However, in this case, the percentage of variant chain exceeds a quarter of the total α-chain.

In summary, we described a new variant Hb Liuzhou, the heterozygote state of which showed clinical and hematological silence. The presence of Hb Liuzhou can be easily identified by MALDI-TOF MS instead of HbA_{1c} procedures based on traditional cation-exchange HPLC and CE.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Patrinos GP, Giardine B, Riemer C, et al. Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res. 2004;32(Database issue):D537–D541.

[2] Huisman TH. Combinations of beta chain abnormal hemoglobins with each other or with beta-thalassemia determinants with known mutations: influence on phenotype. Clin Chem. 1997;43(10):1850–1856.

[3] Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000;46(8 Pt 2):1284–1290.

[4] Klingenberg O, Furuset T, Hestbråten CR, et al. HbA1c analysis by capillary electrophoresis - comparison with chromatography and an immunological method. Scand J Clin Lab Invest. 2017;77(6):458–464.

[5] Strickland SW, Campbell ST, Little RR, et al. Prevalence of rare hemoglobin variants identified during measurements of Hb A1c by capillary electrophoresis. Clin Chem. 2017;63(12):1901–1902.

[6] Little RR, La'ulu SL, Hanson SE, et al. Effects of 49 different rare Hb variants on HbA1c measurement in eight methods. J Diabetes Sci Technol. 2015;9(4):849–856.

[7] Xu A, Chen W, Xia Y, et al. Effects of common hemoglobin variants on HbA1c measurements in China: results for α- and β-globin variants measured by six methods. Clin Chem Lab Med. 2018;56(8):1353–1361.

[8] Zurbriggen K, Schmugge M, Schmid M, et al. Analysis of minor hemoglobins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Chem. 2005;51(6):989–996.

[9] Xu A, Wang Y, Li J, et al. Evaluation of MALDI-TOF MS for the measurement of glycated hemoglobin. Clin Chim Acta. 2019;498:154–160.

[10] Xu A, Lei J, Li J, et al. Falsely high HbA1c value due to a novel α1-globin gene mutation: Hb Shantou [α127(H10)Lys > Glu; HBA1: c.382 A > G]. Scand J Clin Lab Invest. 2018;78(7-8):621–625.

[11] Little RR, Rohlfing CL, Hanson S, et al. Effects of hemoglobin (Hb) E and HbD traits on measurements of glycated Hb (HbA1c) by 23 methods. Clin Chem. 2008;54(8):1277–1282.

[12] Roberts NB, Amara AB, Morris M, et al. Long-term evaluation of electrospray ionization mass spectrometric analysis of glycated hemoglobin. Clin Chem. 2001;47(2):316–321.

[13] Hattan SJ, Parker KC, Vestal ML, et al. Analysis and quantitation of glycated hemoglobin by matrix assisted laser desorption/ionization time of flight mass spectrometry. J Am Soc Mass Spectrom. 2016;27(3):532–541.

[14] Kleinert P, Schmid M, Zurbriggen K, et al. Mass spectrometry: a tool for enhanced detection of hemoglobin variants. Clin Chem. 2008;54(1):69–76.

[15] Moradkhani K, Préhu C, Old J, et al. Mutations in the paralogous human alpha-globin genes yielding identical hemoglobin variants. Ann Hematol. 2009;88(6):535–543.