Quantification of HbA₁c in Patients with Hemoglobin Variants Using Capillary Electrophoresis

A. Aigner, C. Steiner, L. Mustafa

Med. chem. Labor Dr. Mustafa, Dr. Richter OG, Salzburg, Austria

BACKGROUND and OBJECTIVES

Quantification of glycated hemoglobin (HbA₁c) is used to monitor long-term glycemic controls in patients suffering from diabetes mellitus (DM). Guideline goals have been recently established defining the reliability of HbA₁c results regarding the risks for DM complications. Ion exchange High Performance Liquid Chromatography (HPLC), boronate affinity HPLC, immunoassay and enzymatic assays are used to measure of glycated hemoglobin. The accuracy of HbA₁c measurement can be affected by hemoglobin variants, elevated HbF or disorders affecting the lifespan of erythrocytes. Improvement of HPLC methods resulted in modification of analysis time and minimizing analytical interferences. Nevertheless, due to certain hemoglobin variants separation was not performed and HbA₁c results could not be obtained. Separation of glycated hemoglobin in electrophoresis is completely different from HPLC methods. A new capillary electrophoresis system for quantification of HbA₁c was evaluated.

MATERIALS and METHODS

125 blood samples from patients sent for routine follow up investigation were analyzed. Quantification of HbA₁c was done by Capillaries 2 Flex Piercing (Sebia, Lisses). Hba₁c results were obtained and compared to ADAMS A1c HA-8180V (AKRAY France and Japan). Quality assurance tests were in place. 35 blood samples with known heterozygous hemoglobin variants were collected and stored at -20°C for analysis. Hemoglobin variants were verified using Hemoglobin Gel Electrophoresis (Sebia, Lisses).

RESULTS

As shown in Figure 1 statistical significance was verified using the R-squared coefficient calculation to estimate correlation.

Fig.1: Correlation plot with linear regression line

Blood samples containing the most frequent hemoglobin traits HbS, HbD, HbC, HbE and HbA₂ were analyzed. Peaks were separated and no significant interference regarding HbA₁c values was observed. Separation profiles obtained with samples containing elevated rate of HbA₁c are shown in Figure 1 and Hb variants HBE and HBD are shown in Figure 3 and 4. In 4 blood samples presenting rare hemoglobin variants HbA₁c could not be separated and results were not possible.

Fig.2

Fig.3

Fig.4

DISCUSSION

For the vast majority of patients with diabetes, quantification of HbA₁c is a reliable method to assess the risk of developing complications. Altered erythrocyte lifespan and hemoglobin variants concerning the binding of glucose must be considered as inappropriate for glycemic control. The accuracy of HbA₁c values can also be affected by hemoglobin variants, depending on the type of method used for analysis. Potential interference from HbE and HbD traits occurs in ion exchange HPLC and can be observed in the chromatogram. In these cases HbA₁c results are not reported. Using capillary electrophoresis for quantification of glycated hemoglobin in routine laboratory allows for quantification of HbA₁c accurately in patients with the most common hemoglobin variants.