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Effect of HbS in the determination of HbA_2 with the TOSOH HLC-723G7 analyzer and the HELENA Beta-Thal Quik column kit

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Abstract

Objectives: The analytical performance of the TOSOH HLC-723G7 hemoglobin HPLC analyzer and the effect of the presence of HbS in the determination of HbA_2 using HPLC and manual column methods.

Design and methods: The performance characteristics of the TOSOH HLC-723G7 analyzer in the determination of HbA_2 were compared to those of the HELENA Beta-Thal Quik column. The effect of HbS presence in the samples was quantified using the HELENA SAS-MX alkaline gel electrophoresis kit as the reference method.

Results: Within-run and between-run CVs for HbA_2 were better for the TOSOH HPLC analyzer than for the HELENA manual column method. The presence of HbS in the samples produces a strong positive bias in the $\%HbA_2$ values when using both the HPLC and manual column methods, compared to the alkaline electrophoresis gel.

Conclusion: Both the TOSOH HPLC and the manual column are reliable methods for $\%HbA_2$ determination when no HbS is detectable in the samples. When HbS is present, the gel electrophoresis method gives more accurate results.

Keywords: Hemoglobin A_2; Hemoglobin S; HPLC; Method comparison; Gel electrophoresis

Introduction

Measurement of the percentage of hemoglobin A_2 (HbA_2) is a finding of great value in the diagnosis and characterization of hemoglobinopathies, especially in identifying β-thalassemia traits in the screening for β-thalassemia [1]. To this day, a lot of effort is being directed towards the development of analytical methods for the percentage assessment of the various types of hemoglobins in clinical samples, including hemoglobin A_2. The latter is particularly important for the diagnosis of the β-thalassemia trait, in genetic counseling and in the prevention of β-thalassemia. In most cases where the percentage value of HbA_2 is considerably lower or higher than reference levels, it is easy to come to a clinical decision. However, in cases where the values of HbA_2 are in the "grey" or borderline area, as a result of a potential iron deficiency [2], identifying the β-thalassemia trait can be problematic. To this end, an accurate determination would be of valuable clinical use.

Many methods for the determination of HbA_2 levels are available in routine use in the clinical laboratory, such as cellulose acetate electrophoresis [3], anion exchange chromatography in microcolumns [4], HPLC methods [5], ELISA [6], and capillary electrophoresis [7]. An advantage of the HPLC methods is that they can be fully automated, especially when performed on dedicated instruments, as usually is the case.

In this investigation we compare the HELENA Beta-Thal Quik column (an ion exchange microcolumn method for the determination of HbA_2) to the TOSOH HLC-723G7 automated

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HPLC hemoglobin analyzer. We also study the effect of the presence of hemoglobin S (HbS) in the assessment of HbA_2 by HPLC and microcolumn ion exchange chromatography.

Materials and methods

HPLC system

The TOSOH HLC-723G7 automated hemoglobin analyzer is a fully automated HPLC system for the quantification of HbA_2, among other hemoglobins. Hemoglobin separation is performed by a thermostatically controlled cation exchange polymer column (TSKgel G7 β-Thal.HSi, TOSOH Corporation, USA). The separation is achieved during a 7.5-min run using three citric acid buffers with different pH and salt concentrations. The fractions are measured in two wavelengths (415 and 690 nm).

Microcolumn ion exchange chromatography

The HELENA Beta-Thal Quik column (HELENA Biosciences Europe, UK) is a cellulose ion exchange resin, into which the hemoglobins are initially retained, after the addition of hemolyzed red blood cells. The HbA_2 fraction is then eluted first by changing the pH and the salt concentration in the column and measured in a photometer at 415 nm. In parallel, the total hemolysate, containing all classes of hemoglobins, is measured at the same wavelength. The HbA_2 percentage is calculated as the quotient of the HbA_2 fraction absorbance to the absorbance of the total hemolysate.

Alkaline agarose gel chromatography

The HELENA SAS-MX alkaline Hb kit is based on an electrophoretic method for the separation and identification of hemoglobins. The electrophoresis is performed in alkaline buffer. After the separation of the hemoglobins the gel is stained by acid blue stain and the results are obtained after scanning of the gel and integration using the DE400+ densitometer and the accompanying analysis software (Biomidi Electrophoresis, France).

Treatment of samples

Blood samples containing EDTA as anticoagulant were used. Samples were processed from the primary tubes. Calibrators and controls were provided by the manufacturer and handled as directed.

Method comparison

All blood samples were measured in duplicate with each of the two methods. The threshold for within-method duplicate outlier detection was set as four times the mean absolute differences between duplicates for each method and four times the relative absolute differences. Data points that fell outside of both limits were eliminated. The threshold for between-method outliers was set as four times the mean absolute differences between methods and their average and four times the relative absolute differences between methods and their average. Data points that fell outside of both limits were eliminated [8]. In any case, not more than one data point was needed to be eliminated. In the remaining data points, linear regression analysis was performed.

Analytical imprecision

Within-run imprecision was measured by assaying two blood samples, one normal and one from a subject with the β-thalassemia trait, measured twenty times consecutively in succession. Between-run imprecision was measured by assaying two control samples, a low- and a high-value one, in 30 independent runs over 4 months. For the HELENA Beta-Thal Quik column the controls were the HbA2 Quik Column Control-Normal and -Abnormal (HELENA Biosciences, UK). For the TOSOH HLC-723G7 HPLC system the controls used were the Hemoglobin F and A2 Control Set, Low and High level (TOSOH Corporation, USA). Within each run, two aliquots from the same sample were assayed (with an interval of at least 2 h), mixed with patient samples, where appropriate. Outliers were eliminated when the absolute value of the difference of the duplicates exceeded the within-run standard deviation by 5.5 times.

Results

Comparison of the HELENA Beta-Thal Quik column and the TOSOH HLC-723G7 instrument

HbA_2 values measured using the HELENA ion exchange microcolumn (Beta-Thal Quik column) were compared to those measured using the TOSOH HLC-723G7 HPLC system. The linear regression plot is presented in Fig. 1. A relatively good linear correlation was obtained for the two methods ($r=0.975$, $p<0.0001$, 95% CI: 0.963 to 0.983), although for high percentage values of HbA_2 it was found to be somewhat biased.

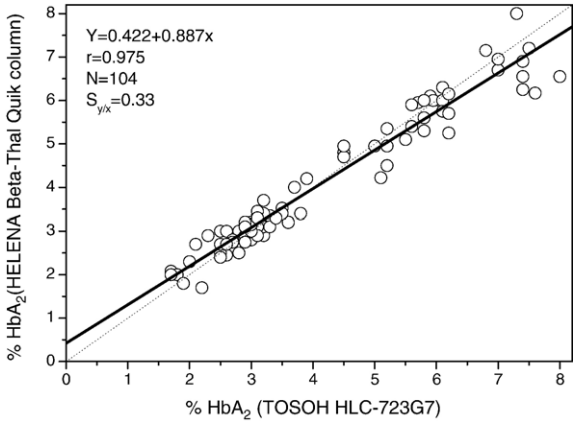

Fig. 1. Linear regression plot for the comparison of the HELENA Beta-Thal Quik column and the TOSOH HLC-723G7 system. The thick solid line is the calculated linear regression line.