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Comparison of Sebia capillary electrophoresis with the Bio-Rad VARIANT II HPLC in the evaluation of HbA2 in diagnosing beta thalassemia

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The guideline for diagnosis of beta thalassemia trait in Sri Lanka defines low red cell indices (MCV<80 fl, MCH<27 pg) in FBC and HbA2>3.5% by quantification. Different cutoffs for HbA2 value are used in other countries (i.e. in India >4%). Thus, the precision of the HbA2 value is crucial for labelling a person as beta thalassemia trait. High-Performance Liquid Chromatography (HPLC) and capillary electrophoresis (CE) are two different techniques for quantifying HbA2 levels. This study aims to compare the HbA2 results of these two systems in individuals with varying HbA2 values and to assess the consistency when repeated of the two systems. The Bio-Rad VARIANT II HPLC (Bio-Rad, Hercules, USA) and the Sebia Capillarys CE (software version 9.3) analyzers were used as directed by the manufacturer. Using normal and pathological quality control materials, we determined the quality parameter, "between day precision", of both analyzers as per CLSI guidelines (EP15-A2 document). EDTA anticoagulated blood samples of patients (203) were analyzed by both methods during a 3-months period. Subjects (100) with HbA2 values between 1.8-3.3% were considered non-beta thalassemic, i.e. normal, while individuals (50) with HbA2 values >4.1% were categorized as beta thalassemia trait. We defined HbA2 levels as borderline (53) if they were between 3.4 and 4.0%. Incompatible FBC patterns and iron deficiency anemia was excluded from each group. Data analysis was performed using SPSS statistical software. HbA2 values by the CE method were slightly but significantly lower than those of the HPLC method, with a mean difference of 0.24 (Paired t-test; $p < 0.001$). Also, HbA2 results by HPLC and CE methods showed a good relationship between each other (Pearson coefficient correlation; r was 0.98). We statistically analyzed this variation and relationship separately among normal, beta thalassemia trait and borderline groups. The variation in HbA2 value was high (mean difference; 0.27) among the normal group, while it was less (mean difference; 0.15) among beta thalassemia traits. The beta thalassemia trait group showed the highest positive relationship (r=0.92). The borderline group showed the least positive relationship (r=0.76). However, both analytical systems showed very close results (CV< 10%) when repeating the same sample between different days. This confirmed the excellent repeatability and acceptability of generated results by both analyzers. In conclusion, HbA2 values obtained from the two methods have a consistent and significant difference in normal, beta thalassemia trait and borderline samples. The variation in HbA2 values between CE and HPLC methods will make the accurate diagnosis of beta thalassemia traits more difficult based on a single reference cutoff value in the borderline group. Therefore, when issuing a diagnosis of beta thalassemia trait in borderline values, this machine-related variation of the HbA2 level should be borne in mind.

Keywords: Beta thalassemia trait, HbA2, Borderline HbA2, HPLC, CE

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