Accounting for Artifactually Elevated HbA₂ in Cases of Hb Hope When Measured by Capillary Electrophoresis

To the Editor

Panyasai et al.¹ point out an important problem with the measurement of hemoglobin (Hb) A₂. By comparing 11 cases of Hb Hope analyzed by capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC; Bio-Rad β-Thalassemia Short Program, Bio-Rad, Hercules, CA), they document a much greater increase in the HbA₂ value measured by CE than by HPLC. They also point out that this increase could lead to an errant interpretation of β-thalassemia trait.

The authors suggest that the increase in HbA₂ could result from a preferential synthesis of HbA₂ because the normal δ chains compete better than the variant Hb Hope β chain for the available α chains. This phenomenon has been reported with other variants.²⁻⁴ However, if this were the explanation for the increased HbA₂ in the present case, the HbA₂ should be increased to a similar extent by HPLC and CE. To explain the considerable difference in HbA₂ results between HPLC and CE, the authors hypothesize a coelution of several Hb Hope adducts with HbA₂.

However, in reviewing the electrophoretogram in their article, we noted that the Hb Hope and HbA overlap in the CE pattern. Because of this, the software shifts the baseline up to approximate the percentage of HbA and Hb Hope. However, while this provides an estimate of the relative amounts of HbA and Hb Hope, it does not account for the overlapping hemoglobin below it in the pattern. Because the percentage of HbA₂ is calculated as a percentage of the total hemoglobin, by not accounting for the overlap area, the percentage of HbA₂ is falsely elevated.

To look at this possibility, we went back to 2 cases of Hb Hope that were diagnosed in our laboratory by CE (Sebia Capillarys², Sebia, Norcross, GA) and confirmed by HPLC (Ultra2 method, Trinity Biotech, Kansas City, MO). The first was a case of Hb Hope similar to the cases reported by Panyasai et al.¹ that had an elevated HbA₂ of 6.0%. Figure 1A, whereas by HPLC, the HbA₂ was 3.1%. However, when we constructed the baseline to include the area under the 2 peaks, the HbA₂ gave a value of 3.4% (Figure 1B), much more comparable to the HPLC because it was now being calculated as a percentage of the total hemoglobin. To see if HbA₂ was elevated in a condition in which there was no overlap with the other β chain product, we examined a case of doubly heterozygous HbS/Hb Hope. As shown in Figure 1C, there was no overlap between the products of the 2 β variants. The HbA₂ was 3.4%.

This consideration complements the important observation that when examined by CE, HbA₂ is artifactually elevated in the presence of Hb Hope. We predict that similar artifactual elevations of HbA₂ could occur in other variant situations in which a large portion of the major hemoglobins involved are excluded by the baseline. By adjusting the baseline in such cases, a false impression of possible β-thalassemia can be avoided.

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References

The Authors’ Reply

We thank Keren and Sample for their comments, which explain that false elevation of the HbA2 level is found any time when 2 major peaks of electrophoresis are in adjoining zones, for example, HbA/Athens-Georgia, S/D Punjab, and S/G-Philadelphia. This explains the false evaluation of HbA2 in our study1 when HbA/Hb Hope peaks were found. The writers advised that false elevation of HbA2 can be manually corrected by taking the nonblack area under both peaks of HbA and Hb Hope (inverted V, the area of mixing HbA and Hb Hope) into account in the analysis system as recommended by the manufacturer (Sebia). We did not have any knowledge or find any recommendation in a Thai instruction received from the distributor in Thailand regarding this manual calculation. As a result, the major peaks in adjoining zones were not taken into account in our study.

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We have now done a manual correction and found that HbA₂ levels decreased to the reference range (1.5%-3.5%) \[\text{Figure 1}\]. Moreover, the mean ± SD of HbA₂ level for the 11 samples was decreased from 4.68±0.60 % (range, 4.0%-6.0%) to 2.02±0.15 % (range, 1.6%-2.4%).

While our manuscript was being reviewed, we found cases of \(\beta\)-thalassemia/Hb Hope. The electrophoretograms in these cases were completely different from those found in cases of Hb Hope with HbA₂ elevations reported in our article. There was no HbA peak in these new findings.

The writer’s explanation of how 2 major peaks of electrophoresis with the adjoining zones affect the HbA₂ level gives us new knowledge. Consequently, all HbA₂ levels were recalculated. Results are stated above.

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Reference

\[\text{Figure 1}\] The capillary electrophoresis electrophoretograms of heterozygous hemoglobin (Hb) Hope before baseline correction (A) and after baseline correction (B) by taking the nonblack area under both peaks of HbA and Hb Hope (inverted V, the area of mixing HbA and Hb Hope) into account in the analysis system.