Higher Sensitivity of Capillary Electrophoresis in Detecting Hemoglobin A2’ Compared to Traditional Gel Electrophoresis

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Abstract. HbA2’ (also called Hb B2) is the most common delta-globin chain defect and is reported to occur in 1-2% of the African American population. The major clinical significance of HbA2’ is that the failure to detect it might lead to an underestimation of the total HbA2, leading to failure to diagnose β-thalassemia minor. In order to diagnose β-thalassemia minor, both HbA2 and HbA2’ levels must be combined. Hb A2’ accounts for a small percentage (1-2%) of the total hemoglobin in heterozygotes. It is difficult to detect this small amount by traditional gel electrophoresis. Using HPLC Hb A2’ is easily detected as it produces a minor peak in the S window. Other conditions which might interfere with detection of HbA2’ by HPLC include Hb S trait or Hb SS disease (Hb A2’ hidden in the S peak), transfused Hb SS (Hb S peak may be very small), Hb C trait or Hb CC disease (glycosylated Hb C elutes in the S window), and Hb G (Hb G2 elutes in the S window). All of the above conditions, including Hb A2’, occur most commonly in the same ethnic group (African American).

We reviewed 654 consecutive cases over a period of three months for the presence of Hb A2’ in our laboratory where capillary electrophoresis is used as the primary diagnostic tool. We detected seven cases (1.07 %) of HbA2’. In contrast, we did not detect any HbA2’ using conventional gel electrophoresis in the last one year (2,580 cases). Although in none of the seven cases the sum of Hb A2 and Hb A2’ exceeded 3.5%, we believe that capillary electrophoresis allows for a better detection of Hb A2’ than gel electrophoresis and HPLC.

Introduction

Hemoglobin (Hb) A2’ (also called HbB2) is the most common delta-globin chain defect and is reported to occur in 1-2% of the African American population [1-8]. Hemoglobin A2’ may be present in homozygous and heterozygous states, as well as in combination with thalassemias [9-11]. The major clinical significance of HbA2’ is that failure to detect it might lead to an underestimation of the total HbA2, leading to failure to diagnose β-thalassemia minor. In order to diagnose β-thalassemia minor, both HbA2 and HbA2’ levels must be combined [5]. Because HbA2’ is such a small percentage of the total Hb in heterozygotes, it can be easily missed when using high-performance liquid chromatography or gel electrophoresis alone. Other conditions which might interfere with detection of HbA2’ by HPLC include Hb S trait or Hb SS disease (Hb A2’ hidden in the S peak), transfused Hb SS (Hb S peak may be very small), Hb C trait or Hb CC disease (glycosylated Hb C elutes in the S window), and Hb G (Hb G2 elutes in the S window). All of the above conditions including Hb A2’ occur most commonly in the same ethnic group (African American). In capillary electrophoresis, Hb A2’ is detected by its presence in zone 1, Hb S is seen in zone 5, Hb C in zone 2, and Hb G in zone 6. Thus, capillary electrophoresis simplifies HbA2’ detection because the HbA2’ elutes in different windows from the major hemoglobins.

Recently, capillary electrophoresis is replacing alkaline electrophoresis as a primary screening method in many hospitals. Capillary electrophoresis has the advantage of decreased manual labor, lower cost, and can fractionate even minor hemoglobin components accurately. Previously, using HPLC to detect HbA2’ was described in the literature by Van...
In capillary electrophoresis, the hemoglobin adducts such as glycosylated HbC (HbC1c) or HbS (HbS1c) are found in the same zone as the parent hemoglobin. This is not the case in HPLC. Thus in capillary electrophoresis, Hb A2’ is detected by its presence in zone 1, Hb S is seen in zone 5, Hb C in zone 2, and Hb G in zone 6.

In 2012, Memorial Hermann Hospital at the Texas Medical Center switched from alkaline electrophoresis to hemoglobin electrophoresis as the primary screening method for hemoglobin identification. We suspected that there was an increase in the amount of cases with a detectable level of HbA2’, which prompted us to review all of the cases retrospectively to determine the prevalence of HbA2’ in our patient population.

**Materials and Methods**

All hemoglobin electrophoreses and corresponding HPLC performed at the Memorial Hermann Hospital at the Texas Medical Center from January 1, 2012 to April 30, 2012 were reviewed, and all new hemoglobinopathy and thalassemia diagnoses were identified. All patient samples with a peak in zone 1 were further studied using HPLC to confirm cases of HbA2’. None of the samples identified with a peak in zone 1 had corresponding peaks in the zones for HbS, HbC or HbG-Philadelphia.

Hemoglobin capillary electrophoresis was performed using the Sebia CAPILLARYS 2 system according to the manufacturer’s instructions. A high voltage protein separation was performed to direct the detection of hemoglobins at 415 nm (which is specific to hemoglobins).
Results

A total of 654 hemoglobin capillary electrophoresis screens were reviewed and 7 new HbA2’ hemoglobinopathy diagnoses were made in this 4 month period, making it the fourth most common hemoglobinopathy condition diagnosed in our patient population following HbS trait, beta-thalassemia minor, and HbC trait. All patients with HbA2’ were African American (7 of 7 cases) and most were female (6 of 7 cases). Most of these samples were African American women of childbearing age, which reflects the local practice of screening all new pregnant mothers of color for hemoglobinopathies.

The HbA2’ trait was considered when a small peak was present in zone 1. For heterozygotes with the HbA2’ trait, the HbA2 levels ranged from 0.9% to 1.8% (mean, 1.3%; SD, 0.32%), and the HbA2’ levels ranged from 0.6% to 1.4% (mean, 1.0%; SD, 0.28%). In all of the cases, (7 of 7), the proportion of HbA2’ was less than the proportion of HbA2. These results are summarized in Table 1. A representative hemoglobin electrophoresis is demonstrated in Figure 1A with corresponding HPLC in Figure 1B. No cases were found to have either double heterozygosity for HbA2’ or Beta-thalassemia minor based on hemoglobin electrophoresis findings; the sum of HbA2 and HbA2’ was not greater than 3.5% in 7 of 7 cases.

Discussion

We reviewed 654 consecutive cases over a period of three months for presence of Hb A2’ in our laboratory where capillary electrophoresis is used as the primary screening tool. We detected seven cases (1.07 %) of HbA2’. In contrast, we did not detect any HbA2’ using conventional gel electrophoresis in the last year (2,580 cases). Although none of the seven cases had a sum of Hb A2 and Hb A2’ that exceeded 3.5%, we believe that capillary electrophoresis allows for better detection of Hb A2’ than gel electrophoresis and HPLC do. Our capillary electrophoresis findings and prevalence estimates concur with the results of Joutovsky et al, who analyzed more than 60,000 samples in an ethnically diverse population (13). The population studied was the metropolitan area of Houston, TX, but it had a selection bias towards pregnant non-Caucasian females and patients undergoing evaluation for anemia which skewed our population sampling since normal males, non-pregnant females and Caucasian pregnant females were under represented.

References


Table 1. Clinical and Hemoglobin Electrophoresis features of 7 patients with hemoglobin (Hb) A2’ trait

<table>
<thead>
<tr>
<th>Feature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>6/7 female</td>
</tr>
<tr>
<td>Race</td>
<td>7/7 African American</td>
</tr>
<tr>
<td>Age range</td>
<td>2 weeks – 69 years</td>
</tr>
<tr>
<td>HbA2%</td>
<td>1.3 (SD, 0.32)</td>
</tr>
<tr>
<td>HbA2’%</td>
<td>1.0 (SD, 0.28)</td>
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