Development of an ELISA strip for the detection of α thalassemias

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Haematologica 2009 [Epub ahead of print]
doi:10.3324/haematol.2009.016592

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Development of an ELISA strip for the detection of α thalassemias

α thalassemia is probably the most common of all single-gene disorders throughout the world. Most incidences of α thalassemia arise from the deletion of one (−) or both (−−) of the α globin genes, which are known as α+ thalassemia (−α/−α) or α− thalassemia (−/−α). The deletion of one α globin gene from one chromosome and two α globin genes from the other chromosome leads to HbH disease (−/−), which is a severe form of α thalassemia. The most severe form of α thalassemia is HB Barts’ hydrops fetalis syndrome, where both α globin genes are deleted (−/−). Previous diagnoses of various types of α thalassemia have been based on clinical pictures, hematological parameters, and percent of HB Bart’s. DNA technology enabled the diagnosis of all different forms of α thalassemia; however, this technology is available only in relatively sophisticated laboratories. More commonly, the differential diagnosis of various types of α thalassemia is determined by measurement of the percentage of Hb Bart’s, which is only semiquantitative.

In previous studies, we produced a highly specific mAb against HB Bart’s, and we developed a corresponding ELISA assay that was able to determine quantitatively the level of HB Bart’s (ng/mL) in hemoglobin solutions of various types of α thalassemia. In this report, we describe the development of a simple ELISA strip (Figure 1) for the detection of HB Bart’s, with the goal of producing a convenient, inexpensive, and dependable assay for the screening of α thalassemia.

The participants in this study included 105 subjects from 39 families with HbH disease 131 pregnant women, and 58 PCR-genotyping negative for α thalassemia. The study was approved by the Institutional Ethics Committee (file no. 275/2549). Analysis by PCR genotyping revealed that for the 105 subjects from the families with HbH disease, 41 had HbH disease, 33 had α+ thalassemia, 12 had α− thalassemia (−3.7 kb), and 19 were heterozygous HbCS; for the 131 pregnant women, 8 had HbH disease, 18 had α− thalassemia, 27 had α+ thalassemia (−4.2 kb), 2 had α− thalassemia (−3.7 kb), 2 had α+ thalassemia (−4.2 kb), and 7 were heterozygous HbCS. In contrast, PCR genotyping of all 58 normal subjects were negative for α thalassemia. When the ELISA strip was used to detect HB Bart’s in the 105 subjects from the families with HbH disease, all were positive. (Table 1). There were 56 out of 131 pregnant women positive to the ELISA strip, while 62 out of 131 women positive to the ELISA strip, while 62 out of 131 pregnant women positive to the ELISA strip. Of 2 having heterozygous HbCS, 24 (92.3%) were positive to the ELISA strip. Of 2 having heterozygous HbCS, 24 (92.3%) were positive to the ELISA strip.

Table 1. Comparison of the ELISA strip and PCR genotyping of α thalassemia.

<table>
<thead>
<tr>
<th>Number of Subjects*</th>
<th>Types of α thalassemia</th>
<th>PCR Genotyping +ve**</th>
<th>ELISA Strip +ve***</th>
<th>%positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>HbH disease</td>
<td>-/α</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>51</td>
<td>α− thal (-3.7 kb)</td>
<td>-α/αα</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>39</td>
<td>α− thal (-4.2 kb)</td>
<td>-α/ααα</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>α− thal (-4.2 kb)</td>
<td>-α/αααα</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>26</td>
<td>Heterozyg. HbCS</td>
<td>αα/ααα</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>127**** Normal</td>
<td>Normal</td>
<td>αα/ααα</td>
<td>8</td>
<td>119</td>
</tr>
</tbody>
</table>

*Number of subjects positive by PCR genotyping = 167 (156 positive but 11 negative by ELISA strip). **Number of subjects positive by ELISA strip = 164 (156 positive but 8 negative by PCR genotyping). ***Number of subjects negative by ELISA strip = 130 (119 negative but 11 positive by PCR genotyping). ****Number of subjects negative by PCR genotyping = 127 (119 negative but 8 positive by ELISA strip). Sensitivity = 165x100/156 = 93.4%; specificity = 119x100/119 = 93.7%; predictive value of a positive test (PV+) = 156x100/164 = 95.1%; predictive value of a negative test (PV–) = 119x100/130 = 91.5%.

Figure 1. Examples of the ELISA strip for the detection of HB Bart’s in hemoglobin solutions of various types of α thalassemia. The ELISA strip was positive when a purple circle was observed on the membrane, and the ELISA strip was negative when no purple circle was observed on the membrane. Sample control means the ELISA strip being treated with all reagents except the hemoglobin solution.
typing used in this study because there were at least 29 deletions that involve both α globin genes. 

The erythrocyte osmotic fragility test (OFT) has been suggested as a simple screening method for α thalassemia and α thalassemia.9,10 When the OFT was compared with PCR genotyping, the sensitivity, specificity, positive predictive value, and negative predictive value of the OFT were 97.7, 74.9, 29.4, and 99.7%, respectively.10

In parts of the world where the incidence of thalassemia is high, the disease causes a tremendous economic loss for the society as a whole. Therefore, the development of a facile and efficient screening program can be readily justified.

The results presented here, which compare our ELISA strip method to standard PCR genotyping of α thalassemia, demonstrate that the ELISA strip test is highly sensitive and highly specific, with excellent predictive values of both positive and negative tests. The ELISA strip test is simple, inexpensive, and requires no sophisticated equipment or expertise; consequently, it can be readily conducted in small diagnostic laboratories. Given these considerations in their entirety, we wish to promote the ELISA strip as a screening test for the detection of α thalassemia in the general population.

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Funding: this work was supported by a grant from the National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, and the Faculty of Medicine Endowment Fund, Faculty of Medicine, Chiang Mai University, Thailand.

Acknowledgments: The authors would like to thank Professor Dr. T. Randall Lee, University of Houston, Texas, USA for revising our manuscript.

Keyword: α thalassemia, Hb Bart’s, ELISA strip, monoclonal antibody

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