Hb Bart’s Level in Cord Blood and Deletions of α-Globin Genes

By Luan Eng Lie-Injo, Arnasalam Solai, Alejandro R. Herrera, Leigh Nicolaissen, Yuet Wai Kan, Wong Pui Wan, and Khalid Hasan

The white blood cell DNA of 36 cord blood samples with Hb Bart’s in the red blood cells was studied for α-globin gene deletions by hybridization of DNA fragments digested by the restriction endonucleases Eco RI, Hpa I, Bam HI, and Bgl II. All 16 DNA samples from cord blood with Hb Bart’s below 3% and no other abnormal hemoglobin had one α-globin gene deletion (αthal1), except one which had two α-globin gene deletions (αthal1). Most of the αthal1 were of the rightward deletion αthala genotype. Two new types of αthal2 variation were found, probably due to a polymorphism somewhere in an area outside the α-globin gene. All 16 DNA samples from cord blood with Hb Bart’s between 3.5% and 8.5% and no other abnormal hemoglobin had two α-globin gene deletions (αthal1), except one that did not have any α-globin gene deletion and one that had one α-globin gene deletion. Sixty-five DNA samples from cord blood without Hb Bart’s or other abnormal hemoglobin had no α-globin gene deletions, except one that had one α-globin gene deletion (αthal1). Two of the 65 DNA samples were found to have triplicated α-globin gene loci.

TWO TYPES OF α-thalassemia, one severe and one mild, have long been recognized in Asians. The homozygous condition for the severe type of α-thalassemia, clinically recognized as Hb Bart’s hydrops fetalis,1 is due to deletion of all α-globin genes.2 Hb Bart disease is the result of the combination of the severe and mild type of α-thalassemia and is due to a deletion of three of four α-globin genes, which indicates that the α-globin gene is duplicated. In Asians, the trait for the severe type of α-thalassemia is usually associated with deletion of two α-globin genes (mostly on the same chromosome) and that for the mild type with deletion of one α-globin gene. A nondeletion type α-thalassemia has recently been reported.5

The severe and mild type of α-thalassemia designated as α-thalassemia, (αthal1) and α-thalassemia, (αthal2), respectively,4 are thought to be represented in the newborn period by an appreciable or by a trace amount of Hb Bart’s, respectively. Earlier studies showed that the frequency of Hb Bart’s in the newborn period is rather high in Malaysia.7 The present study is an attempt to correlate the level of Hb Bart’s in the newborn period with the presence and degree of α-globin gene deletion and possibly with other abnormalities associated with the α-globin genes. For this purpose a new survey of cord blood in Malaysian newborns of different racial groups was carried out.

MATERIALS AND METHODS

Collection of Study Material

Cord blood samples were collected in acid citrate dextrose (ACD) solution from the delivery room of the Maternity Unit, General Hospital, Kuala Lumpur, Malaysia, and processed within 24 hr. Part of the blood was separated for hemoglobin analysis, and white blood cells were isolated from the remaining blood by hemolyzing the red cells and precipitating the white cells by centrifugation. The white blood cells were dissolved in Tris-EDTA(TE) buffer, quick frozen, and kept at −70°C. The white cell batches were air freighted in dry ice to San Francisco where they were kept at −70°C until further processing.

Normal placenta for isolation of normal cellular DNA was collected from the delivery room of Moffit Hospital, University of California, San Francisco.

Hemoglobin Analysis

Hemoglobin analysis was carried out in Kuala Lumpur, Malaysia, by starch-gel electrophoresis at pH 8.6 and the cord blood samples found to have Hb Bart were analyzed further; Hb Bart’s was quantitated by the cellulose acetate electrophoretic method.

Preparation of DNA and Digestion With Restriction Enzymes

DNA was prepared from white blood cells by phenol-chloroform extraction and ethanol precipitation as described before.6 DNA was digested by restriction endonuclease Eco RI, Hpa I, Bam HI, and Bgl II under conditions recommended by the manufacturers from whom the nuclease was obtained.

Hybridization Patterns of DNA Restriction Enzyme Fragments

DNA fragments obtained by restriction endonuclease digestion were electrophoresed in 0.8% agarose gel and transferred to nitrocellulose filter paper according to Southern8 with modifications. The DNA fragments in the filter paper were hybridized with...
\[3^2\text{P}-\text{labeled cDNA probe synthesized by reverse transcription of purified mRNA obtained from reticulocyte-rich adult blood or from blood of newborns obtained at exchange transfusion as described before.}^3\]

\[\text{Where indicated, the DNA fragments were also hybridized with specific }3^2\text{P}-\text{labeled }\alpha\text{-globin gene probe prepared by nick translation as previously described}^4\text{ from }\alpha\text{-globin gene cloned in plasmid JW 101,}^5\text{ a gift from Dr. B. G. Forget. Hybridization was carried out for 2–3 days, followed by thoroughly washing the nitrocellulose filter under stringent conditions and autoradiography for 2–5 days.}\]

**Recognition of athal, Trait on Autoradiograms by Densitometry**

The restriction endonuclease Hpa I cuts human DNA outside, and between, the two \(\alpha\)-globin genes, producing two DNA fragments carrying \(\alpha\)-globin genes (Fig. 1). On a Hpa I hybridization pattern, using a probe derived from an adult patient with high reticulocyte count and increase of fetal hemoglobin, the \(\alpha\)-globin genes are located on fragments of 14.5 kb and 4.2 kb in length (Figs. 1 and 2), whereas the \(\gamma\)-globin genes are located on fragments 24 kb and 4.8 kb in length (Fig. 2). In \(\alpha\)-thal, trait, the 14.5 kb Hpa I fragment carrying the \(\beta\)'-\(\alpha\)-globin genes and the 4.2 kb Hpa I fragment carrying the \(\delta\)'-\(\alpha\)-globin genes both are 1 \(M\) as opposed to 2 \(M\) in normals. We estimated the molarity of \(\alpha\)-globin genes located on the fragment of 4.2 kb in length by comparing the density of the \(\alpha\)-globin gene band divided by the density of the \(\gamma\)-globin gene band in each sample with the density of the \(\alpha\)-globin gene band divided by the density of the \(\gamma\)-globin gene band in a normal control on the same autoradiogram, using the \(\gamma\)-globin gene bands as internal controls. This \(\gamma\)-globin gene band in different autoradiographic films may differ in intensity depending on the level of \(\gamma\)-cDNA present in the total cDNA probe used for hybridization. Comparisons with normal controls must therefore be carried out on the same autoradiogram. Densitometric scanning was carried out on a Helena Quick Scan Flur-Vis and the relative values for \(\alpha\)- and \(\gamma\)-globin gene bands were automatically recorded on a Helena Quick Quant II. The density of the \(\alpha\)-globin gene band divided by the density of the \(\gamma\)-globin gene band in normal DNA was arbitrarily chosen to represent the value of two \(\alpha\)-globin genes. The density of the \(\alpha\)-globin gene band divided by the density of the \(\gamma\)-globin gene band in the sample to be examined on the same autoradiogram film is then compared with the value obtained for two \(\alpha\)-globin genes in the normal control (see Fig. 3). In different control \(\alpha\)-thal, trait carriers (parents of hydrops fetalis and \(HbH\) disease patients), the number of \(\alpha\)-globin genes located on the 4.2-kb Hpa I fragment is around 1.
Table 1. Hb Bart's Levels in Cord Blood of Malaysian Newborns

<table>
<thead>
<tr>
<th>Racial group</th>
<th>Chinese</th>
<th>Malay</th>
<th>Indian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number examined</td>
<td>323</td>
<td>344</td>
<td>176</td>
</tr>
<tr>
<td>With Hb Bart's level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5%</td>
<td>9</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>3.5%-8.5%</td>
<td>11</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>3.3%-4.2% + Hb CoSP</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3.0%-4.5% + other abn. Hb</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>22.0%-26.0%</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Not examined</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total numbers with Hb Bart's</td>
<td>24</td>
<td>25</td>
<td>9</td>
</tr>
</tbody>
</table>

i.e., they are molar as opposed to 2M in controls (see Table 3). In athal2, the 4.2-kb Hpa I fragment (however, not the 14.5-kb Hpa I fragment) also carries one-half the normal number of α-globin genes, but athal1 trait can easily be differentiated from athal1 trait on an Eco RI hybridization pattern where in athal2 trait, there are two α-globin gene carrying fragments, one normal, one faster-moving, whereas in athal1, there is only one normal fragment carrying α-globin genes. It must be said, however, that this method of quantitation is valid only if the bands in the autoradiogram are well defined with densities within the reliable range and the autoradiogram has a clear and even background. In order to achieve this, the blot hybridization has sometimes to be repeated.

RESULTS

Hemoglobin analysis was carried out in 843 newborns (323 Chinese, 344 Malays, and 176 Indians) and 58 of them were found to have Hb Bart's. The results are shown in Table 1.

A total of 36 DNA samples were studied by restriction endonuclease digestion and blot hybridization analysis. The results are summarized in Table 2. We also studied 65 DNA samples from cord blood that did not contain Hb Bart's or any other abnormal hemoglobin.

DNA of Cord Blood With Hb Bart's Below 3% Without Abnormal Hemoglobin

Digestion of normal DNA by Eco RI produces a single α-globin gene specific fragment 23-kb long, and digestion of normal DNA by Bam HI produces a single α-globin gene specific fragment 14.5-kb long.

All but one of the 16 DNA samples of cord blood with Hb Bart's level below 3.0% and not accompanied by an abnormal hemoglobin showed two α-globin gene bands in the Eco RI restriction endonuclease hybridization pattern as expected for athal1 trait condition13,14 (Table 2). One fragment is 23-kb long, the other was a faster moving fragment. We found two types of this faster moving fragment (Table 2). One variant with a fragment of 19 kb in length (found in 11 DNA samples) has been described earlier.13,14 The other type had a fragment that moved slightly slower and is about 21 kb long (found in 4 samples) which has not been reported before (Fig. 4). Interestingly, however, while in the Eco RI gene patterns there are two types of abnormal band, on Bam HI gene patterns the two variants show the same abnormal 11.5-kb fragment. This may indicate that the difference between the two variants is due to a base change in the 3' Eco RI site, removing the recognition sequence and bringing in the next 3' downstream Eco RI site. Further studies are required to confirm the polymorphism and to identify the Eco RI site involved.

One DNA sample of cord blood with a trace amount of Hb Bart's showed on Eco RI and Bam HI hybridization patterns only one normal α-specific fragment. Quantitative estimation of the α-globin genes located on the 4.2-kb Hpa I fragment as described in Materials and Methods, showed about half the normal

Table 2. Sizes of α-Specific Fragments Obtained After Restriction Endonuclease Digestion of the DNA From Newborns With Different Levels of Hb Bart in Their Hemolysate

<table>
<thead>
<tr>
<th>Hb Bart's Level</th>
<th>No. Studied</th>
<th>Eco RI</th>
<th>Bam HI</th>
<th>Hpa I</th>
<th>Bgl II</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5%</td>
<td>16</td>
<td>(11)*</td>
<td>(4)</td>
<td>(1)</td>
<td>(14)</td>
</tr>
<tr>
<td>3.5%-8.5%</td>
<td>14</td>
<td>(13)</td>
<td>(1)</td>
<td>(13)</td>
<td>(1)</td>
</tr>
<tr>
<td>3.3%-4.2% + Hb CoSP</td>
<td>3</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>22.0%-26.0%</td>
<td>2</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>Not examined</td>
</tr>
<tr>
<td>80%</td>
<td>1</td>
<td>Gene absent</td>
<td>Gene absent</td>
<td>Gene absent</td>
<td>Not examined</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses are numbers of individuals.
HB BART'S AND α-GLOBIN GENE DELETIONS

Fig. 4. Autoradiogram of Eco RI endonuclease digestion patterns of human DNA. Total globin cDNA derived from a patient with sickle cell anemia was used as probe. Lanes 1, 3, 4 and 6: normal. Lane 5: athal, usual type (19 kb). Lanes 2 and 7: athal1 new variant (21 kb). The three fastest-moving bands have been cut off from the picture.

Table 3. Number of α-Globin Genes on 4.2-kb Hpa I Fragment Based on 2 Being the Normal Number (the 4.8-kb γ-Specific Hpa I Fragment Was Used as Internal Control)

<table>
<thead>
<tr>
<th>Control</th>
<th>Newborns with Hb Bart's &lt; 3.5%</th>
<th>Newborns With Hb Bart's 3.5% - 8.5%</th>
<th>Newborns With Hb Bart's + Hb CoSp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nos.</td>
<td>No. of α-Genes</td>
<td>No. of α-Genes</td>
<td>No. of α-Genes</td>
</tr>
<tr>
<td>1</td>
<td>1.05</td>
<td>1</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>0.96</td>
<td>2</td>
<td>0.93</td>
</tr>
<tr>
<td>3</td>
<td>0.90</td>
<td>3</td>
<td>1.08</td>
</tr>
<tr>
<td>4</td>
<td>0.94</td>
<td>4</td>
<td>1.12</td>
</tr>
<tr>
<td>5</td>
<td>0.89</td>
<td>5</td>
<td>1.83</td>
</tr>
<tr>
<td>6</td>
<td>1.08</td>
<td>6</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>1.09</td>
<td>7</td>
<td>1.02</td>
</tr>
<tr>
<td>8</td>
<td>1.05</td>
<td>8</td>
<td>1.24</td>
</tr>
<tr>
<td>9</td>
<td>0.88</td>
<td>9</td>
<td>1.12</td>
</tr>
<tr>
<td>10</td>
<td>0.96</td>
<td>10</td>
<td>0.90</td>
</tr>
<tr>
<td>11</td>
<td>0.78</td>
<td>11</td>
<td>1.33</td>
</tr>
<tr>
<td>12</td>
<td>1.28</td>
<td>12</td>
<td>0.64</td>
</tr>
<tr>
<td>13</td>
<td>0.99</td>
<td>13</td>
<td>1.12</td>
</tr>
<tr>
<td>14</td>
<td>0.89</td>
<td>14</td>
<td>0.93</td>
</tr>
<tr>
<td>15</td>
<td>1.12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.93</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Mean: 0.99
SD: 0.12

*Nos. 1 through 9 were parents of babies with hydrops fetalis. Nos. 10 through 15 were parents of patients with HbH disease.

†The autoradiographic pattern of several samples (those with a dash) were not suitable for scanning because the background of the autoradiogram was not even or contained black spots, or the density of the bands was not within the reliable range. However, the blot hybridization was not repeated because two α-specific bands were clearly seen on the autoradiogram of Eco RI restriction enzyme patterns, establishing the diagnosis of athal trait.

number of α-globin genes to be present (Table 3, no.12). This is probably a case of athal1, that showed a level of Hb Bart's that overlaps with that seen in athal1.

One DNA sample had an abnormal α-specific Bam HI fragment not 11.5 kb long, as the other samples did, but an even faster-moving fragment about 10.0 kb long. That all the fast-moving, abnormal bands described above carried α-globin genes was demonstrated by hybridization with the specific 32P-labeled α-globin gene probe, prepared by nick translation of α-globin DNA from α-plasmid JW 101.

Embry et al. demonstrated that the single α-globin gene deletion in the athal genotype is of two kinds. In the so-called leftward-deletion athal genotype, the deletion involves the 5′α-globin gene, whereas in the rightward-deletion athal genotype, the deletion possibly involves both normal α-globin genes, probably the result of nonhomologous, unequal, crossing over. The rightward-deletion athal1 genotype has also independently been defined as a crossover variant of athal1 by Phillips et al. The rightward-deletion athal2 and leftward deletion athal1 genotypes can be differentiated from each other by analysis of the DNA fragments obtained by Bgl II restriction endonuclease digestion.

The result of our present study shows that the rightward deletion athal2 genotype is more common in Malaysia. Of those examined on Bgl II gene maps (Fig. 5), 6 of 8 Chinese, 3 of 4 Malays, and 3 of 3 Indians had the rightward athal2 genotype. One sample could not be examined by Bgl II digestion because supply was exhausted.
DNA of Cord Blood With Hb Bart's Between 3.5% and 8.5%

All but one of the 14 DNA samples of cord blood with Hb Bart's between 3.5% and 8.5% had one normal α-specific Eco RI and Bam HI fragment (Table 2). The exception had two α-specific Eco RI fragments 23 kb and 19 kb long and two α-specific Bam HI fragments 14.5 kb and 11.5 kb long. This case is compatible with the diagnosis athal1 trait.

This group with appreciable Hb Bart's, thought to be athal1 trait carriers, had only one α-specific Eco RI and Bam HI fragment just like the normal controls, in which there is no gene deletion. Therefore, they have to be differentiated from the normals. This was carried out by densitometric scanning of the α- and γ-globin gene bands on Hpa I gene patterns, as described under Materials and Methods. The results showed that, except for one, all DNA samples had only about one-half the normal number of α-globin genes on the 4.2 kb Hpa I fragment (Table 3). One exception had the normal number of α-globin genes on the 4.2-kb Hpa I fragment (no. 5 in Table 3). The results show that Hb Bart's between 3.5% and 8.5% in Asian newborns is associated with the athal1 trait condition whereby two α-globin genes are deleted on one chromosome. One was an athal2 instead of athal1, trait carrier. The other exception in this group could be a case of a nondeletion type α-thalassemia or a case of a Hb CoSp carrier in whom the Hb CoSp was not detectable at birth. In Hb CoSp carriers there is no α-globin gene deletion and the gene pattern looks normal (see the next group and Table 3).

DNA of Cord Blood With Hb Bart's Accompanying Hb CoSp

Three DNA samples of cord blood with Hb Bart's level ranging from 2.1% to 3.7% accompanying the presence of Hb CoSp showed a normal pattern on the Eco RI, Bam HI, Hpa I, and Bgl II gene maps. Quantitation of α-globin genes in the 4.2-kb Hpa I fragment showed the normal number of α-globin genes, which indicates that there is no α-globin gene deletion in this condition.

DNA of Cord Blood With 22.0% and 25.6% Hb Bart's

The two DNA samples, one with 22.0% and one with 25.6% Hb Bart in cord blood, showed only one abnormal, fast-moving band at 19 kb in length on the Eco RI gene map and a missing 4.2-kb band on the Hpa I gene map. This is expected in HbH disease where three of four α-globin genes are deleted.4

DNA of Cord Blood With Almost All Hemoglobin Being Hb Bart's (Hb Bart's Hydrops Fetalis)

One DNA sample from cord blood of a stillborn hydrops fetalis baby with almost all hemoglobin being Hb Bart's was found to be devoid of α-globin genes as expected.

DNA of Cord Blood Without Hb Bart's

In all 65 samples (18 Chinese, 30 Malays, 17 Indians) without Hb Bart's or other abnormal hemoglobin, the DNA had only one α-specific Eco RI fragment of 23 kb in length, as expected, except for the DNA of three individuals. One of the 3 (Chinese) showed an additional faster-moving α-specific fragment 19 kb long, compatible with the diagnosis of athal1 trait. Densitometric scanning of an autographic Hpa I hybridization pattern (see Materials and Methods) in this case confirmed the diagnosis of athal1 trait. Apparently, this case is indeed an athal1 carrier, even though Hb Bart's was not detectable in the cord blood. In the second one (Malay), there was an additional α-specific Eco RI fragment, slightly slower-moving than the normal 23-kb band. It was only resolved from the normal α-specific Eco RI fragment when the run was more prolonged. The third exception (Chinese) also had a slow-moving α-specific fragment clearly separated from the normal 23-kb fragment. This abnormal fragment moved even slower than the slow-moving fragment found in the second case above. Detailed studies of these last two cases by restriction endonuclease digestion and by hybridization analysis using different enzymes showed them to be carriers of two types of triplicated α-globin loci. One of these two variants is identical with the one reported earlier.17,18 A detailed description of the two types of triplicated α-globin loci encountered in the present study has been reported elsewhere by Lie-Injo et al.19

DISCUSSION

It has long been recognized that the presence of Hb Bart's in the Asian newborn is associated with α-thalassemia. However, the exact molecular basis has not been demonstrated. Some opinions held that the presence of Hb Bart's in blacks may also be due to a metabolic imbalance of hemoglobin chain synthesis in the newborn period.20 In recent studies in blacks, Higgs et al.21 found that all their cases with Hb Bart's in the newborn period had one or two α-globin genes deleted. They also found that deletion of one α-globin gene may not be associated with a detectable amount of Hb Bart's at birth. Ohene-Frempong et al.22 found that, although most black newborns with Hb Bart's have one of two α-globin genes deleted, many who
were thought to have the normal number of four α-globin genes may also have a trace amount of Hb Bart’s in the range found for cases with three α-globin genes. It is not clear, however, how the normal number of α-globin genes was determined in those cases.

It has further been observed that Hb Bart’s in the newborn can accompany the synthesis of different structurally abnormal hemoglobins.\(^{21}\) This led to the opinion that although the Hb Bart’s in general is associated with α-thalassemia, it is not specific and may sometimes be due to a nonspecific imbalance of chain synthesis accompanying the synthesis of certain structurally abnormal hemoglobins.

The results of the present study show that, with the exception of those associated with Hb CoSp, a structurally abnormal hemoglobin, a trace amount of Hb Bart’s (<3%) in Malaysian newborns indicates one α-globin gene deletion, an appreciable amount of Hb Bart’s (3.5%–8.5%) is associated with two α-globin gene deletions (usually on one chromosome), a high level of Hb Bart’s is associated with deletion of three α-globin genes, and hydrops fetalis with the hemoglobin being almost all Hb Bart’s is associated with deletion of all α-globin genes.

Earlier findings of Hb Bart’s in the newborn accompanying structurally abnormal hemoglobin synthesis, HbQ\(^{\ast}\), Hb CoSp, Hbf\(_{KL}\), and HbE\(^{23}\) may be evaluated as follows. The gene for HbQ\(^{\ast}\) has recently been shown to neighbor an α-globin gene deletion,\(^{14}\) and therefore, the finding of Hb Bart’s in the newborn accompanying HbQ is not surprising. Hb CoSp is not accompanied by an α-globin gene deletion. However, Hb CoSp is regarded as a kind of α-thalassemia because there is an imbalance in the α-chain to non-α-chain synthesis associated with Hb CoSp synthesis, and the presence of Hb Bart’s in the newborn is the expression of this imbalance of α- to non-α-globin chain synthesis. The presence of Hb Bart’s occasionally accompanying the synthesis of HbE in the newborn may be due to an association of HbE with α-thalassemia, which should not be rare in Malaysia where both abnormalities are prevalent.\(^{24}\) The production of Hb Bart’s in the newborn accompanying most cases with Hbf\(_{KL}\), a γ-chain mutation,\(^{23}\) is at present still unexplained, because the frequency of Hbf\(_{KL}\), which occurs only in Indians, is low, just like the frequency of α-thalassemia is low in Indians.

As noted earlier,\(^{8,25}\) there is very little overlap between the group with a trace amount of Hb Bart’s (in our study below 3%) and that with an appreciable amount of Hb Bart’s (in our study between 3.5% and 8.5%) usually associated with αthal\(_2\) and αthal\(_1\), respectively. In our study, only one case of αthal, had a Hb Bart’s level in the range usually found for αthal\(_1\), and one case of αthal\(_2\) had a Hb Bart’s level in the range usually found in αthal\(_1\).

Embry et al.\(^{15}\) found 5 among 13 Chinese with HbH disease associated with the nondeletion type of α-thalassemia. Excluding the cases associated with Hb CoSp, we found all cases with Hb Bart’s in the newborn are associated with a deletion of the α-globin gene or genes, except for one case. This one case may be a nondeletion type α-thalassemia, but it may as well be a case of Hb CoSp carrier not detected at birth, because Hb CoSp is difficult to detect in the carrier especially in the newborn period. In an earlier study of 17 families with HbH disease in Malaysia,\(^{26}\) it was found that in more than half the families the HbH was associated with Hb CoSp (at that time designated by Lie-Injo’s group as X component).\(^{26}\) This was also the case in the group of Chinese families.

Two types of αthal\(_2\) have been described that apparently are produced by a different mode of unequal crossing over.\(^{15,16}\) In our present study, we found another common type of variation that is probably due to a polymorphism of DNA located outside the α-globin gene sequences. We also found a single case with a deletion slightly larger than usually seen in the common types of αthal\(_2\) mentioned above (10-kb Bam HI fragment instead of 11.5 kb, see Table 2). This variant is however, not the same as the variant described by Pressley et al.,\(^{27}\) which is due to a deletion of α\(_2\)-gene and part of α\(_1\)-gene, because in our case only one α-globin gene is involved in the deletion, not showing an abnormal band on an Hpa I gene pattern.

In the group of DNA samples from cord blood without Hb Bart’s, we found one case of αthal\(_2\) due to one α-globin gene deletion. We failed to detect the Hb Bart’s in only this one case among 16 cases of αthal\(_2\) we studied. Studies among blacks suggest that many black αthal\(_2\) heterozygotes do not have detectable levels of Hb Bart’s at birth.\(^{21}\) However, methods for the detection of Hb Bart’s in the blood have differed among various studies. We used highly concentrated hemolysates in order not to miss the presence of small hemoglobin components, especially Hb CoSp, which can easily be overlooked in the trait condition,\(^{28}\) especially in the newborn period.

**ADDENDUM**

The diagnosis of αthal, has now been established by blot hybridization of restriction endonuclease digested DNA fragments with specific 32P-labeled γ-globin gene probe prepared by nick translation of γ-globin gene cloned in plasmid (a gift from Dr. Tom Maniatis). The results will be reported elsewhere together with the results of other studies.
ACKNOWLEDGMENT

We thank the staff of the Delivery Room of the Maternity Unit, General Hospital, Kuala Lumpur, Malaysia, for their generous help in collecting cord blood samples from newborns and Dr. B. G. Forget for the gift of JW 101 plasmid containing α-globin genes. The AMV reverse transcriptase was obtained from the Division of Cancer Cause and Prevention, National Cancer Institute.

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Hb Bart's level in cord blood and deletions of alpha-globin genes

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