Neonatal screening for sickle haemoglobinopathies in Birmingham

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Abstract

During 1978–81 there were about 43 500 births in Birmingham, of which 10.3% were to Negroes and 22.6% to Asians. Cellulose acetate electrophoresis of red cell haemolysates from capillary specimens collected for phenylketonuria screening was performed to assess the feasibility, cost, and benefits of detecting sickle haemoglobinopathies early.

Eight babies had important haemoglobinopathies; four were homozygotes for haemoglobin S (HbS), three were mixed heterozygotes for HbS and haemoglobin C (HbC), and one had haemoglobin E (HbE) and β-thalassaemia. Also, 534 (1.19%) were heterozygotes for HbS or haemoglobin D (HbD) and 205 (0.46%) for HbC or HbE, 453 (1.01%) were heterozygotes with a fast-moving band, one was a heterozygote for haemoglobin Norfolk, and one was a heterozygote for both HbS and haemoglobin G Philadelphia.

The cost of neonatal screening for haemoglobinopathies was 12.5p per baby (£0.05 for each serious abnormality).

Introduction

Many studies have shown the incidence and geographical distribution of haemoglobinopathies.1, 2 In populations where sickle haemoglobinopathies are a major health problem early detection may allow genetic counselling of heterozygotes and prevent complications and sudden death in homozygotes.3, 4 Neonatal screening is not, however, a worthwhile procedure in populations with a low incidence of haemoglobinopathies.5 In a survey of immigrant schoolchildren (60% Negroes, 40% Asians) in Birmingham6 8–4%, had a haemoglobin abnormality, the most frequent being haemoglobin S (HbS) (4.7%). The gene frequencies of haemoglobin S and C (Hbc) in Negro children were approximately 8% and 3% respectively and of haemoglobins D (Hbd) and E (Hbe) in Asians 1–4% and 0–5% respectively.

During 1978–81 births in the City of Birmingham rose from 13 599 to 15 324 a year of which about 10–3% were of Negro

References

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and 22.6% of Asian origin. Since 1968 a screening programme for phenylketonuria and other amino acid disorders, using capillary blood,4 has been in progress in the laboratory. As only the plasma was required for the amino-acid chromatography, we were able to undertake a pilot study in 1978 using the red cells from these specimens to assess the feasibility and cost of detecting sickle haemoglobinopathies early, of providing suitable care for infants with haemoglobin phenotypes SS, SC, and SThal, and of counselling their families.

Subjects and methods

Specimens of capillary blood were collected from babies aged 6-14 days by midwives, either at the children's homes or in hospitals, and delivered to the laboratory the same day. The plasma was tested for amino acid disorders and, since 1980, congenital hypothyroidism (unpublished data) while the cells were used to detect HBs and other haemoglobinopathies. After centrifugation in a microhaematocrit centrifuge the section of the tube containing red cells was cut off and vortexed with distilled water in Alpha Laboratories Vortexer to produce haemolysate. Haemolysates (20 per run) were separated by cellulose acetate electrophoresis using a Tris-EDTA boric acid-barbitone buffer system pH 8.6 and stained with Ponceau S. The electrophoretic strips were read by two people independently. Babies with fetal haemoglobin (HbF) and an abnormal band or bands but with no adult haemoglobin (HbA) (likely to be homozygotes for an abnormal haemoglobin or mixed heterozygotes) were seen at a haemoglobinopathy clinic with their parents and family for clinical assessment, blood counts, starch and agar gel electrophoresis, and quantification of HbA2 and HbF.10 In two cases blood was sent to Professor H Lehmann for structural investigation. Heterozygotes were not recalled to the clinic.

HBs and HBc were moved to the same position on cellulose acetate, as did the slower moving HbC and HbE. Confirmation of the haemoglobinopathy was usually possible therefore only if the baby was investigated further in the haemoglobinopathy clinic; in a few cases stored haemolysates were subjected to starch gel electrophoresis to detect small amounts of HbA not visible on cellulose acetate. The ethnic origins of the babies were not recorded on the metabolic disease survey request forms and so were ascertained only in those infants who attended the clinic. Nevertheless, previous studies and population data1 4 indicate that these abnormal bands are usually HBs and HBc in Negro and HbD and HBe in Asian babies. Fast-moving abnormal bands, which were probably HB Barts or fast-moving abnormal haemoglobins, were investigated further in only one infant.

Some babies screened in the neonatal period subsequently attended the hospital for a variety of reasons and were screened for sickle-cell disorders or investigated for thalassaemia. Thus, the results of the neonatal screening and of the subsequent tests could be compared in these babies.

We analysed the cost of screening for haemoglobinopathies, excluding the cost of collecting the capillary blood specimens and transporting them to the laboratory, as this was already part of the phenylketonuria screening procedure. The cost of investigating further the small number of suspected homozygotes and mixed heterozygotes was not estimated as they attended an existing clinic and their tests represented a small proportion of the haemoglobin investigations at the hospital.

Results

Between April 1978 and March 1981 approximately 43 500 babies were born in the city and 44 720 screening tests were performed. The extra tests were accounted for by the screening of babies resident in the city who were born elsewhere and by duplicate tests performed during the investigation for phenylketonuria. The screening tests indicated that 4.19% of babies were heterozygotes for HBs or HBcD, 0.46%, were heterozygotes for HBc or HBcE, and 1.01% had a fast-moving band. These results (table I) were expected from the gene frequencies in the previous Birmingham study.6

The eleven babies who were probably homozygotes for HBs or HBcD (patients 1-7) or double heterozygotes for HBsD and HBcC/E (patients 8 and 9) and one other (patient 11) who had a distinct fast-moving band, attended the haemoglobinopathy clinic for further investigations (table II). Two babies proved to be HbS5 and two (cases 3 and 4), who could not be reviewed, were probably also homozygotes, as their stored haemolysates showed only HbF and HBs or HBd (no HBA) when subjected to starch gel electrophoresis and they were of Negro origin. The necropsy in case 5, on a premature dizygotic twin, showed acute bronchitis and only occasional deformed red cells in small blood vessels; her sickle-cell anaemia was unlikely to have contributed to her death. Starch gel electrophoresis on stored haemolysates showed that one patient (case 5) probably had HbAS. One patient (case 6) had HbAS and one (case 7) HbAD.

### Table I: Results of screening tests for haemoglobinopathies by electrophoresis on cellulose acetate

<table>
<thead>
<tr>
<th>Haemoglobinopathy</th>
<th>No expected from gene frequency</th>
<th>No found</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2 or AD</td>
<td>510</td>
<td>534</td>
</tr>
<tr>
<td>A2 or AE</td>
<td>97</td>
<td>205</td>
</tr>
<tr>
<td>Fast-moving band</td>
<td>Not known</td>
<td>453</td>
</tr>
<tr>
<td>SS or DD</td>
<td>Not known</td>
<td>27</td>
</tr>
<tr>
<td>S/D with C/E</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CC or ED</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>Distinct fast-moving band</td>
<td>Not known</td>
<td>1</td>
</tr>
<tr>
<td>Abnormal band between S and C</td>
<td>Not known</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>Not known</td>
<td>43 514</td>
</tr>
</tbody>
</table>

*Includes β-thalassaemia, HbA, variants, and other rare Hb variants.

### Table II: Results of further investigation of haemoglobin abnormalities

<table>
<thead>
<tr>
<th>Patient</th>
<th>Haemoglobin abnormality by screening test</th>
<th>Racial origin</th>
<th>Final diagnosis and comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SS or DD</td>
<td>Negro</td>
<td>SS</td>
</tr>
<tr>
<td>2</td>
<td>SS or DD</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>3</td>
<td>SS or DD</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>4</td>
<td>SS or DD</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>5</td>
<td>SS or DD</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>6</td>
<td>SS or DD</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>7</td>
<td>SS or DD</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>8</td>
<td>S/D with C/E</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>9</td>
<td>S/D with C/E</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>10</td>
<td>S/D with C/E</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>11</td>
<td>S/D with C/E</td>
<td>Negro</td>
<td>S/D</td>
</tr>
<tr>
<td>12</td>
<td>Fast-moving band</td>
<td>Caucasian AA</td>
<td>Hb Norfolk heterozygote</td>
</tr>
</tbody>
</table>

All three babies of Negro origin suspected to have HbS/D with C/E were confirmed to be HbSc double heterozygotes. One infant (case 11) a Caucasian, had no haemoglobinopathy, however, and repeat electrophoresis of her stored neonatal red cell haemolysates was normal; the error probably occurred because the haemolysate had been unsatisfactorily applied to the cellulose strip. In case 12 the patient was the daughter of an Italian father and English mother, and both the baby and her mother, whose ancestors came from Birmingham (so far as could be traced), were heterozygotes for Hb Norfolk (αS Gly −Asp). One other baby, on neonatal screening was thought to be HbCC or HbEE (table I), proved to have HbE and β-thalassaemia (Bβ Thal). This girl, an Asian, was transfusion-dependent. Haemoglobin electrophoresis does not distinguish between HbEE and Eβ Thal.

The results of haemoglobin investigations in 84 babies who attended the hospital after their initial neonatal screening procedure are shown in table III. β-Thalassaemia cannot usually be detected in the neonatal period by haemoglobin electrophoresis, but two babies with HbAS were also not detected. No homozygotes are known to have escaped

### Table III: Results of haemoglobin investigations performed in the neonatal period compared with subsequent findings

<table>
<thead>
<tr>
<th>Haemoglobinopathy</th>
<th>Initial tests</th>
<th>Subsequent tests</th>
<th>Failure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>17</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>AC</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Heterozygous β Thal</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heterozygous β Thal</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eβ Thal</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heterozygous for both HbS and HbG Philadelphia</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AA</td>
<td>53</td>
<td>57</td>
<td>4</td>
</tr>
</tbody>
</table>

*Previously interpreted as an abnormal band with mobility between HbS and HbC.
Discussion

Garrick et al. concluded that screening for sickle-cell anaemia was economically worthwhile if 10% of the population were Negroes. Now that about one-third of babies born in Birmingham are Negroes or Asians, screening all babies for haemoglobinopathies is more cost effective than recording ethnic origins and identifying those blood specimens which should be tested. Among the Asian population the principal haemoglobinopathy of clinical significance is $\beta$-thalassaemia, which cannot be reliably identified by neonatal haemoglobin electrophoresis, although $\alpha$-thalassaemia, which may produce equally severe anaemia, is detectable and was found in one baby.

During the first three years neonatal screening of the Birmingham population yielded eight important haemoglobinopathies (four HbSS, three HbcS, and one HbS/Thal)—that is, one per 5590 tests. These figures compare favourably with the detection rate in our laboratory for phenylketonuria (1/23 000) and hypothyroidism (1/3000). When linked with screening for metabolic errors the additional cost of identifying each baby with a serious haemoglobinopathy was about £705.

The number of heterozygotes, homozygotes, and mixed heterozygotes was close to that expected from the known gene frequencies. The number of babies recalled for further investigations, who ultimately proved to be heterozygotes, was acceptably low (four), although two families were anxious until the final diagnosis, when reassurance could be given. Others have reported that occasionally it is difficult to distinguish HbAS from HbSS on neonatal blood when using cellulose acetate. We agree with this observation; when there was any doubt about whether the baby was a heterozygote or a homozygote because of the small amount of HbA present the child was recalled for further investigations. Repeat electrophoresis on blood from babies who subsequently attended the hospital when most of their fetal haemoglobin had been replaced by adult haemoglobin gave results which correlated well with the neonatal screening tests and thus confirmed the reliability of the method. No examples of the rarer mixed heterozygotes, hereditary persistence of fetal haemoglobin and haemoglobin $S$ $\beta$-thalassaemia ($S$ $\beta$ Thal), were detected, although both occur in the Birmingham Negro population. Since their neonatal electrophoresis patterns are identical to those of HbSS children, we should detect any babies born with these disorders in future. Thus, we have found that in the neonatal period all babies with clinically important sickle haemoglobinopathies can be detected, while causing little anxiety to parents of heterozygotes.

Other workers have performed citrate agar electrophoresis (pH 6.2) to distinguish HbS from HbD heterozygotes but have used benzidine to stain the haemoglobins. Our technique is similar, but since benzidine is unavailable in Britain because of haemoproteic properties, we use naphthol black. With this it is not always possible to detect the small amounts of HbS present in heterozygotes during the first weeks of life, and we are now working on a technique, using tetra-methylbenzidine, which may prove more sensitive and help in differentiating between heterozygotes and homozygotes for HbS.

We had agreed not to contact or investigate further heterozygotes since to do so, without a major public health education programme, would create unnecessary distress and no benefit to those concerned. Apart from two families who emigrated shortly after the births of their babies, we had no difficulty in contacting babies who needed further investigation or in obtaining the permission of their parents.

The purpose of detecting sickle haemoglobinopathies early is to aid rapid diagnosis and treatment of potentially fatal complications and to provide families with genetic counselling. Among Jamaican babies diagnosed by cord血 electrophoresis and followed prospectively, 13% of children with HbSS and 5% with those with HbSC, compared with one of unaffected control children, died within the first two years of life, mostly from acute splenic sequestration and pneumococcal septicaemia. Powars reported that over 10 years seven babies with apparent "sudden infant death syndrome" had HbSS and pneumococcal septicemia on Hb electrophoresis and blood culture at necropsy. Nevertheless, in a prospective study of 98 Birmingham children with sickle haemoglobinopathies, there were only five deaths, all of children aged more than 3 years; three had originally presented with pneumococcal meningitis at 1, 0, 1, and 2 weeks but all had recovered. Some babies with undiagnosed sickle haemoglobinopathies may have died of apparent "sudden infant death syndrome." In this study case 3 was therefore of particular interest, although the necropsy findings indicated that this patient's sickle-cell anaemia probably did not contribute to her death; very few reports of deaths attributable to complications of sickle-cell anaemia at such a young age have been published.

The babies with sickle cell haemoglobinopathies detected by neonatal screening in Birmingham are being followed up and an assessment of any benefits of early diagnosis will be reported.

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