Neonatal screening for haemoglobinopathy

Results in 7691 Manchester newborns

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Evans, D. I. K., and Blair, V. M. (1976). *Archives of Disease in Childhood*, 51, 127. **Neonatal screening for haemoglobinopathy: results in 7691 Manchester newborns.** Over a period of one year the blood samples collected for phenylketonuria testing from 7691 Manchester newborns were screened by haemoglobin electrophoresis. An abnormality was detected in 47 (0.61%) of the babies. No cases of homozygous haemoglobinopathy were found. The overall incidence of sickle-cell trait was 0.38%, but for the Black population it was 10%. Four Black babies and one White baby had alpha-thalassaemia. No other haemoglobinopathies were found in the White babies and no Asian baby had alpha-thalassaemia. Haemoglobin A2 was precociously developed in three babies, two of whom were coloured—probably a further example of the earlier maturity of coloured babies.

The screening programme was stopped when it became clear that the incidence of disease was low, but testing for abnormal haemoglobins could easily be combined with screening for metabolic disease in places where the incidence of haemoglobinopathies is higher.

All babies in the United Kingdom are screened for phenylketonuria, the incidence of which is about 1 in 7000 (Komrower, 1974). The haemoglobinopathies occur much more often in susceptible populations. Many surveys for abnormal haemoglobins among adults have been made in different parts of the world, but relatively few have been made among babies. Immigrants to the large industrial cities of Britain have brought with them a number of genes for abnormal haemoglobins but the true incidence is unknown. We therefore screened Manchester babies as an extension of the existing programme of screening for phenylketonuria and other metabolic diseases to try to detect sickle-cell disease early and to find the incidence of abnormal haemoglobins.

Subjects and methods

In the Manchester region blood samples are collected by health visitors into heparinized microcapillary tubes from all newborns between the ages of 10 and 14 days in hospital and at home (Sardharwalla *et al.*, 1972). The samples are sent to the Royal Manchester Children's

Hospital, where they are spun in a microhaematocrit centrifuge (Hawsley, London). The plasma is used to screen for phenylketonuria and other aminoacidurias by one-way chromatography (Komrower, 1974). Between March 1973 and February 1974 the packed cells from the samples of 7691 newborns were sent to the haematology department and used for haemoglobin electrophoresis on cellulose acetate sheets (Celagram, Shandon, London) in a Tris-EDTA-borate buffer pH 8.9 with a Shandon U 77 tank and power pack. The method was based on that described by Kohn (1969) modified by Barnes, Komarmy, and Novack (1972).

If the screening test showed an abnormality a venous sample was obtained from the baby by a doctor from the local authority health department and sent in by post together with blood samples from the parents and other children. In some cases the health visitors called on the mother at home to ask her to bring her baby and other children to the hospital, where the blood was taken and the baby examined. In all cases the patient's general practitioner was first contacted, usually by telephone, and the investigation and its implications for the family explained. The general practitioner was often able to provide helpful details about the baby such as its racial origin, its health and that of its family, the attitude of the mother, and the family background.

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The blood samples were used for haemoglobin (Hb) estimation, reticulocyte counts, and a repeat Hb electrophoresis to confirm the abnormality. Other tests such as Hb solubility, a sickling test, tests for Hb H, alkali denaturation, and acid elution were done as indicated. When Hb S was detected in a baby a diagnosis of homozygous sickle-cell disease was excluded if the mother's Hb was normal or if agar gel electrophoresis in citrate buffer pH 6.2 (Robinson *et al.*, 1957) showed Hb A with F and S.

Most (6912) of the blood samples were from babies born in Manchester, but 505 were from babies born in the neighbouring borough of Stretford and 274 were from babies living in Manchester but born elsewhere.

Results

In 12 months of screening 7691 heel-prick samples were tested, of which 56 (0.73%) showed an abnormality. The babies were retested, and an abnormal Hb pattern was confirmed in 47 (0.61%). At this time 146 family members were tested. 58 were abnormal and 88 were normal. The mothers of the 47 babies were checked but only 16 (29%) of the fathers, as many were unable or unwilling to be tested and some were unknown. Details are shown in the Table.

TABLE

Haemoglobin abnormalities found in 47 newborn babies out of 7691 screened and in 58 out of 146 relatives of the affected babies. In addition three babies had adult levels of haemoglobin A2.

	Babies	Relatives	Total
Homozygotes			
CC	0	1	1
Heterozygotes			
AS	29	39	68
AD	4	7	11
AC	7	7	14
AE	1	1	2
G-Philadelphia	1	1	2
α -thalassaemia	5	2	7
Total	47	58	105

The commonest abnormality was the sickle-cell trait, found in 29 babies (0.38%). Seven babies had Hb C. Four had Hb D: three were of Asian (D-Punjab) and one of West African origin (D-Ibadan). In addition, a Chinese baby with Hb E and an Indian baby with an α -chain variant, probably G-Philadelphia, were found. No baby had sickle-cell disease, and the only homozygote was a mother with Hb-C disease. All these babies were children of coloured immigrant parents: no White baby had an abnormal slow band.

Five babies showed a fast band of Hb Bart's on initial screening between the 10th and 15th day of life, but in none was it confirmed in a blood sample taken about 2 months later, although one showed some Hb H cells, as did two family members. 4 were Negro: one was White. Black babies comprised only about 3% of the total sample, so the incidence of Hb Bart's in Blacks was 1.4%—similar to that reported by Weatherall in Baltimore (1963). None of the Asian babies had Hb Bart's. The presence of Hb Bart's was very temporary, but the babies were probably carriers of α -thalassaemia. The α -thalassaemia gene in Blacks is characterized by only slight reduction in α -chain synthesis (Schwartz and Atwater, 1972), and rapid disappearance of Hb Bart's, in one case in 6 days, has also been noted (Minnich *et al.*, 1962; Van Baelen *et al.*, 1969).

Three babies seemed to have more Hb A2 than other newborns. Their first test was done between day 1 and day 16. One weighed 3150 g and another 3400 g at birth and both were boys. In these 2 children a second sample showed a normal pattern: the A2 level had not increased abnormally. No details or second sample from the third baby were available. Whereas in most newborns the A2 band is weak in these three babies it was obvious enough to raise the possibility that there was a trace of a minor component such as Hb C or E. The subsequent sample showed no change in the intensity of the A2 band, and we concluded that Hb A2 was precociously developed in these babies. The parents of one baby and the mother of another had normal blood pictures with no evidence of β -thalassaemia minor or other disorder. One baby was White, 2 were coloured (one Negro, one Asian).

The remaining 6 babies showed an abnormal pattern initially but no abnormality could be confirmed. In 3 cases no abnormality was found in mother or baby: the fathers were not tested. 2 babies were normal at the second test but neither parent was tested. One mother refused to allow a second sample to be collected from her baby.

In only one family was a haemoglobinopathy found in both parents—one had Hb S and the other Hb C. The baby had sickle-cell trait. No baby presented with sickle-cell disease or other homozygous disorder within 21 months after screening stopped, although possibly a case may have escaped detection. Thirteen of the mothers of the 29 babies with sickle-cell trait were normal: the others had sickle-cell trait. Only 5 of the fathers of babies with sickle-cell trait were tested. 3 were normal and 2 had sickle-cell trait. Sickle-cell

disease was excluded in 13 babies with Hb S whose mothers were normal. In the other 16 babies whose mothers had sickle-cell trait agar gel electrophoresis showed Hb A with F and S, thus confirming the carrier state.

Racial origins of babies. The racial origin of all the children tested was not known. The Race Relations Act, 1968, was introduced to reduce racial discrimination, and racial origins are not recorded when blood samples are collected. Racial origin was inquired into only for the babies who needed a second test. The distribution of different races may be inferred from the Census of 1971 and from information available to the school authorities. Most immigrants from the new Commonwealth registered in the Census had come into the United Kingdom after 1945 (Kohler, 1974). Only 3% of the Manchester population in 1971 had been born in the new Commonwealth and the numbers born in India and Pakistan, including Bangladesh (6435), and in Africa and the West Indies (6715) were about equal.

There were 102 000 children in school in Manchester in 1973, of whom 6342 (6%) were listed as immigrants (*Manchester Evening News*, 1973). Assuming that schooling is from the age of 5 to 16, the number of children entering school each year is one-eleventh of the total. Each year about 9300 children start school, of whom 580 would be immigrants. Assuming no great change in the figures, this should be the number of children born five years earlier. The racial distribution would be the same as that of their parents, so half would be of Asian and half of Negro (African and West Indian) origin. Thus there might be about 290 Negro babies born in Manchester each year, or 3.1% of the total. Most of the parents come from the West Indies, particularly from Jamaica, where the incidence of Hb S is 6-9% (World Health Organization, 1966). So from this incidence in 290 Negro babies we should expect 17 to 26 to be affected. This is fewer than the number of our babies (29) who had Hb S. These calculations entail a number of assumptions, but cases are probably not underestimated and our number was probably about what should have been expected.

Discussion

Like most large English industrial cities, Manchester has a growing immigrant population introducing a variety of genes for abnormal haemoglobins. That we found so few haemoglobin abnormalities is surprising. It must be because

the immigrants, though noticeable because of their colour, are still only a small minority. Because they have arrived only recently the genes they bring have not merged with the genetic constitution of the indigenous Mancunians. The small number of carriers explains the absence of homozygous sickle-cell disease. Twenty-nine cases of sickle-cell trait in an estimated 290 Negro babies is 10%, or 5% in all the immigrant babies. In Birmingham, England, Stuart *et al.* (1973) found a 4.5% incidence of sickle-cell trait in immigrant schoolchildren, of whom 40% were of Asian and 60% of African or West Indian origin. The total incidence of abnormal haemoglobins in their 6835 coloured children was 8.4%, and the ratio of Hb S to C was 3 to 1. In Manchester the ratio is 4 to 1, rather higher than the ratio of 2 to 1 given by the World Health Organization (1966) for Jamaica, where most of our affected babies' parents were born.

The incidence of Hb Bart's overall was only 0.07% but for the calculated black population it was about 1.7%. This is similar to the incidence in St. Louis, Missouri, reported by Minnich *et al.* (1962), but lower than the 5% reported by Schneider *et al.* (1974) for black Americans in Texas, and of 17.9% reported for black Congolese in Kinshasa by Van Baelen *et al.* (1969). Such changes must reflect the varying origins of Negroes from Africa, just as does the varying incidence of Hb S and C. Raven and Tooze (1973) pointed out that α -thalassaemia is not uncommon in immigrants in London, but the significance of Hb Bart's in cord blood has been a subject of discussion. Weatherall (1963) suggested that it indicated α -thalassaemia, but Esan (1970) proposed that in Black babies it was a temporary developmental abnormality. The problem has been partially solved by Friedman *et al.* (1974). Of their 690 Black American babies 15% had some Hb Bart's while 3% had over 2% Hb Bart's and showed conclusive evidence of α -thalassaemia later. Children with less than 2% Hb Bart's were not studied. Very small amounts of Hb Bart's are not detected by our screening method, and we regard all the positive cases as having α -thalassaemia.

The precocious development of Hb A2 was not unexpected. Minnich *et al.* (1962) noted that the cord blood from some Negro babies had A2 levels near to those of adults. It is noteworthy that two of our 3 babies were coloured (one Negro, one Asian). This change, rather than the presence of Hb Bart's, ought rightly to be regarded as a harmless developmental abnormality. We cannot explain why it should apparently be more common in non-White babies, unless it is a further example

of the greater maturity at birth of coloured babies.

One large problem was how to explain to parents the meaning of an abnormality. Few know what sickle-cell disease is. Some cannot believe that there is nothing wrong with the baby when we keep testing blood samples. It is impossible to get informed consent for a blood test if parents cannot understand its purpose. As none of our babies was found to have a serious disease none was followed up. Therefore some investigations were not done because we believed that more blood tests would only increase parental anxiety without helping the baby. We have outlined these problems elsewhere (Evans, 1974). Many of the fathers were not tested because the mothers were not married.

Nearly all of the abnormal haemoglobins were found in coloured babies. The only abnormalities in the White babies were a Hb Bart's band in one and early development of Hb A2 in another. These findings contrast with those of Schneider *et al.* (1974) in about the same number of American 'White' babies of whom 76 showed an abnormality, including 61 with haemoglobin Bart's and 8 with Hb S or C. Their findings reflect the mixed racial background of an American population. By the evidence of our study the White population of Manchester has very few genes for haemoglobin disorders.

The pilot trial was discontinued after a year because the incidence of abnormalities was too low to justify its continuance. The original purpose was to detect sickle-cell disease and other serious disorder, but because so few carriers have been detected we do not expect to find more than an occasional homozygote or double heterozygote. In addition, the people do not understand the significance of abnormal haemoglobins, and we believe that such a programme would be justified only in areas where haemoglobinopathies are common and a significant number would be found.

Nevertheless, this method of screening for haemoglobinopathies could be particularly effective when coupled with a programme of screening for metabolic disease. Phenylketonuria screening on cord blood samples is not satisfactory, and blood needs to be taken several days after birth when phenylalanine rises to diagnostic levels. However, in newborns haemoglobin screening is probably easier with cord blood as the samples are larger than can be obtained with heel-prick, making confirmation of an abnormality easier. With the exception of the rapid disappearance of Hb Bart's, there seems to be no change in the haemoglobin pattern in the first few days of life. In parts of the world where haemoglobinopathies are common

and screening for metabolic disease is performed a combined programme of this sort might be very effective.

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