\( \alpha \) Thalassemia and the Hematology of Homozygous Sickle Cell Disease in Childhood


\( \alpha \) Thalassemia modifies the hematologic expression of homozygous sickle cell (SS) disease, resulting in increased total hemoglobin and HbA\textsubscript{2}, and decreased HbF, mean cell volume, reticulocytes, irreversibly sickled cells, and bilirubin levels. The age at which these changes develop in children with SS disease is unknown. Ascertainment of globin gene status in a large representative sample of children with SS disease has afforded an opportunity to study the hematologic indices in nine children homozygous for \( \alpha \) thalassemia 2 (two-gene group), 90 children heterozygous for \( \alpha \) thalassemia 2 (three-gene group), and 167 children with a normal \( \alpha \) globin gene complement (four-gene group). The two-gene group had significantly lower mean cell volumes from birth, higher red cell counts from one month, lower reticulocytes from three months, and higher HbA\textsubscript{2} levels from one year, as compared with the four-gene group. Children with three genes had intermediate indices but resembled more closely the four-gene group. Differences in total hemoglobin or in fetal hemoglobin between the groups were not apparent by eight years of age. The most characteristic differences of the two-gene group were the raised proportional HbA\textsubscript{2} level and low mean cell volume, the latter having some predictive value for \( \alpha \) thalassemia status at birth.

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MATERIALS AND METHODS

Patients. The patients were part of a cohort study of sickle cell disease based on screening of cord blood samples and prospective follow-up of all affected children. During the period from June 1973 to December 1981, 100,000 consecutive normal deliveries were screened at the main Government maternity hospital (Victoria Jubilee Hospital) Kingston, Jamaica, with the detection of 314 children with SS disease, 201 with sickle cell-hemoglobin C (SC) disease, and 47 with the two types of sickle cell-thalassemia.

Methods. Diagnostic procedures included electrophoresis on cellulose acetate and agar gel. The diagnosis of SS disease was made by the finding of hemoglobin bands in the position of Hb S, F, and A\textsubscript{2}. Family studies and the development of HbA\textsubscript{2} levels differentiated children with sickle cell-hereditary persistence of fetal hemoglobin and with sickle cell-\( \beta \) thalassemia. Hematologic procedures and methods of follow-up were as previously described. \( \alpha \) Thalassemia status was determined by restriction endonuclease analysis of DNA obtained from the buffy coat of peripheral blood samples. Currently, results are available in 266 children with 9 (3%) homozygous for \( \alpha \) thalassemia 2 (\( \alpha-/\alpha- \)), 90 (34%) heterozygous for \( \alpha \) thalassemia 2 (\( \alpha-/\alpha \)) and 167 (63%) with a normal \( \alpha \) globin gene complement. Of the 48 SS children without results, 40 were lost before analysis (37 deaths, three emigrated), 6 had defaulted since birth, and 2 children remained to be analyzed. The 266 children with known \( \alpha \) globin genotype therefore represented 97% of all surviving SS children in the cohort study. The number of children contributing data to each genotype fell with advancing age (Table 1) because of the age structure of the cohort study.

Hematologic data were entered on a computer, and analysis was undertaken within age groups defined with increasing limits of tolerance as age-related changes became less marked. The birth age group was confined to data collected over 1 to 3 days, 3 months (± 1 week), 6 months (± 2 weeks), and 1 year (± 4 weeks). Analysis was limited to steady-state data by excluding hematologic values within 90 days of transfusion; during significant clinical illness (serious infection, acute splenic sequestration, aplastic crisis, painful crisis); or in hypersplenism.

Computer programs calculated mean and SD for each variable at each age group. Data from both sexes were combined in the analysis. The distribution of HbF from one year and of reticulocytes from birth were positively skewed and the logarithmic transformations, log\(_{10}\) (percentage of HbF + 4) and log\(_{10}\) (reticulocytes + 1), were used to obtain more normal distributions. Differences between hematologic indices in the genotypes were compared using Student's \( t \) test.

RESULTS

Fetal hemoglobin (HbF) fell sharply over the first six months and more gradually thereafter (Fig 1), there being no consistent differences between the different groups. HbA\textsubscript{2} levels increased with age (Fig 2) and were consistently and significantly higher in the three-gene group and highest in the two-gene group, the difference between the two-gene and the four-gene groups being significant at the 0.1% level throughout.

Total hemoglobin levels fell rapidly over the first three months and more slowly thereafter (Fig 3), and no consistent differences occurred between the groups. Mean cell hemo-
globin concentration (MCHC) varied considerably, lowest values tending to occur in the two-gene group and highest values in the four-gene group, but the differences were not consistent or significant. Red cell counts fell sharply over the values in the four-gene group, but the differences were not group and highest values occurred in the four-gene group, gradually (Fig 5). Lowest values occurred in the two-gene and significantly (P < 0.001) higher in the two-gene as compared with the four-gene group. Values in the three-gene and four-gene groups were generally similar, although they tended to be lower in the four-gene group. Mean cell volume (MCV) declined with age to six months and then rose gradually (Fig 5). Lowest values occurred in the two-gene group and highest values occurred in the four-gene group, the differences being consistent and significant (P < 0.01) at birth and at all ages. A similar pattern was observed with mean cell hemoglobin.

Reticulocyte counts fell sharply from birth and increased steadily after one month to 36 months. Mean values were lower in the two-gene group as compared with the four-gene group at all ages although the difference was only intermittently significant. Values in the three-gene group were intermediate but similar to those in the four-gene group although a significantly (P < 0.05) lower count did occur at six months. When expressed as absolute reticulocyte counts, values were much more variable and, after logarithmic transformation, levels were significantly lower (P < 0.05) in the two-gene as compared with the four-gene group at five years only. No genotype differences occurred in platelet counts or serum iron levels.

In view of the significant differences in MCV between the \( \alpha \) globin genotypes at birth, the value of this index in predicting the \( \alpha \) globin genotype was examined. The ranges of MCV at birth in the two-, three-, and four-gene groups were 79 to 95: n = 4, 89 to 115: n = 21, and 91 to 120: n = 48 respectively, indicating lower values in the two-gene group but almost identical distribution in the three- and four-gene groups. The 95th percentile (calculated from mean ± 1.64 SD) of the two-gene group was 100 fl and the fifth percentile of the 3-gene group was 87 fl indicating a probability of 0.05 that an infant with an MCV of >100 fl would be, and a similar probability than an infant with an MCV of <87 fl would not be homozygous for \( \alpha \) thalassemia 2. Applying this to the 22 children of unknown \( \alpha \) globin genotype in whom values for MCV were available at birth, levels exceeded 102 fl in 20 children and were 97 fl in the remaining two, indicating a low probability that any of these individuals were of the two-gene type.

**DISCUSSION**

These observations on the evolution of the hematological changes in SS disease according to \( \alpha \) thalassemia status are based on substantial numbers of patients. The 266 children represented 85% of the entire group with SS disease diagnosed at birth and 97% of those surviving. Thirty-seven children died before suitable diagnostic procedures became available, but it is unlikely that this loss seriously biased the prevalence of \( \alpha \) thalassemia genotypes since the observed relative proportions agree closely with that predicted from the gene frequency and with figures derived from an older Jamaican population. This loss of children with unknown \( \alpha \) globin genotypes renders this data set unsuitable for analysis of survival, although it is adequate for observing the development of hematologic changes within the different subgroups.

Some of the hematologic characteristics of \( \alpha \) thalassemia emerge early, significantly lower volumes for MCV and MCH being present at birth. In homozygotes, the red cell...
count was significantly higher at one month, reticulocytes were lower at three months, and HbA2 was higher at one year of age. It is apparent therefore that most of the hematologic differences characteristic of α thalassemia are apparent from early in life.

Hematologic features in older populations with α thalassemia which have not yet emerged in the cohort study include differences in MCHC, total hemoglobin, and HbF. There was a consistent trend for lower values of MCHC to occur in children with α thalassemia, but because of the variability of values, this trend was not significant. No difference in total hemoglobin levels was apparent by the age of eight years in contrast to the consistently and significantly elevated levels in α thalassemia in previous reports.1,4 This difference is unexplained, although since values for MCHC and MCV were still below levels for their respective genotypes in adults, it is possible that the difference in total hemoglobin will emerge as these indices stabilize in older children. Another possible explanation is that in older patients the difference in total hemoglobin levels was only significant between the two-gene group and those with three or four genes.4 The difference between levels in three- and four-gene groups in that study was small and not significant, and the lack of expected difference in the present study may be due to the small numbers of patients in the two-gene group.

Data on HbF levels in older populations are conflicting. A small study with five patients homozygous for α thalassemia suggested a significant increase in HbF levels,3 and the recent report from the American Cooperative Study showed a slight but nonsignificant increase in the 13 patients with homozygous α thalassemia diagnosed by gene mapping.4 In contrast, a much larger Jamaican study of 44 patients with homozygous α thalassemia, with carefully matched controls, indicated significantly lower values.4 The magnitude of the reduction was small and was postulated to result from decreased F cell selection secondary to a lower hemolytic rate. Because evidence from reticulocyte counts was consistent with less hemolysis in the present study also, the reason for a lack of difference in HbF by the age of six years is unknown.

It appears that the effects of interacting α thalassemia are generally identifiable within the first few months of life. There is evidence for a reduced hemolytic rate with decreased reticulocyte and increased red cell counts, although no differences in total hemoglobin or HbF are apparent by age eight years. The effect of reduced α globin production is manifest by reduced MCV and MCHC, both of which are theoretically likely to diminish intravascular sickling.11 The overall pattern of the hematologic features seen in Jamaican SS children with homozygous α thalassemia 2 appears similar to that in children with sickle cell β thalassemia,13 whereas evidence from older patients suggests that their clinical course is similar.4

Clinical data derived from the α globin genotypes in the Jamaican sickle cell cohort study is still at an early stage of collection and analysis. The α globin genotype did not appear to influence the age at which specific symptoms of the disease developed16 and the effects of alpha thalassemia on splenic complications are complex. There is a suggestion that acute splenic sequestration may be less common in the two-gene group,17 but preliminary unpublished observations suggest that such patients may be more prone to hypersplenism, consistent with observations in older patients.4 There is controversy on the role of α thalassemia in survival in SS disease; Mears et al18 noted a significant excess of three-gene individuals in older patients, whereas Higgs et al19 were unable to show an increasing prevalence of two-gene individuals with age. These observations could be consistent if the survival benefit of three-gene individuals was compromised by the higher hemoglobin level or other features characteristic of the two-gene group. Unfortunately, because of the unknown α globin genotypes of many of the early deaths, the cohort study cannot contribute to this controversy in relation to early mortality.

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