Hemoglobin E: a common hemoglobinopathy among children of Southeast Asian origin

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With the recent immigration of Southeast Asians to Canada, hemoglobin E has become a frequent diagnosis. The clinical and hematologic findings in 42 children (mean age 4.3 years) with hemoglobin E are presented. There were 33 heterozygotes (having hemoglobin E trait), 6 homozygotes (having hemoglobin EE) and 3 double heterozygotes (having hemoglobin E–β-thalassemia). The heterozygotes had low-normal hemoglobin levels and mean corpuscular volumes; coexisting iron deficiency, present in 62% of these children, resulted in substantially lower hemoglobin levels, very low mean corpuscular volumes and lower than expected levels of hemoglobin E on electrophoresis. The children with hemoglobin EE were only slightly anemic, but those with hemoglobin E–β-thalassemia had severe anemia and required long-term transfusion therapy. Nutritional factors and parasitic infestations were the main causes of iron depletion, which was common, particularly in children less than 2 years old (87%). Physicians of patients of Southeast Asian origin should be aware of the clinical and hematologic presentation of these hemoglobinopathies.

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Depuis l'immigration récente au Canada d'Asiatiques du Sud-est, on y reconnaît souvent l'hémoglobinopathie E. Nous en présentons les signes cliniques et hématologiques chez 42 enfants dont l'âge moyen est 4,3 ans. Ils comprennent 33 hétérozygotes (porteurs de l'hémoglobine E), 6 homozygotes (porteurs de l'hémoglobine EE) et 3 doubles hétérozygotes (porteurs de l'hémoglobine E-β-thalassémie). Chez les hétérozygotes l'hémoglobinémie et le volume globulaire moyen se situent à la limite inférieure de la normale; lorsqu'ils sont également carencés en fer, ce qui est la cas pour 62% d'entre eux, l'hémoglobinémie est nettement plus basse, le volume globulaire moyen très petit et, à l'électrophorèse, la concentration de l'hémoglobine E moindre que la concentration attendue. Si l'anémie des homozygotes est légère, celle des doubles hétérozygotes E-β-thalassémie est grave et nécessite des transfusions étalées sur une longue période. Parmi les enfants âgés de moins de 2 ans, 87% présentent une carence martiale dont les causes sont surtout diététiques et parasitaires. Le médecin qui est appelé à soigner des Asiatiques du Sud-est doit se familiariser avec les signes cliniques et hématologiques de ces hémoglobinopathies.

Hemoglobin E is a structurally abnormal hemoglobin resulting from the substitution of glutamic acid by lysine at position 26 of the β-chain.[1,2] On routine alkaline electrophoresis it migrates with hemoglobin C at the hemoglobin A_2 position. On citrate agar electrophoresis, however, it migrates toward the cathode, with a mobility similar to that of hemoglobin A, whereas

hemoglobin C moves alone toward the anode.[1,2]

Hemoglobin E is one of the most common hemoglobin variants in the world.[1,3] The prevalence of its gene is particularly high (30% to 50%) in areas of Kampuchea, Laos and Thailand.[3-7] Before 1979 this hemoglobin variant was rarely seen in Canada. However, with the increasing number of Southeast Asian immigrants it has become a relatively common diagnosis. At the Children's Hospital of Eastern Ontario, Ottawa, we diagnosed hemoglobin E in 42 patients between 1975 and 1986. In this report we present the clinical and hematologic findings in these patients.

Methods

The clinical and laboratory findings in the 42 children with E hemoglobinopathies were reviewed. Complete blood counts were performed with the Ortho Model ELT-800 (Ortho Instruments, Westwood, Massachusetts). Hemoglobin electrophoresis was done at an alkaline pH (8.4) with cellulose acetate (Beckman Microzone System, Beckman Instruments, Inc., Palo Alto, California) before 1985 and with agarose gel (Corning Electrophoresis System, Corning Medical and Scientific, Medfield, Mass.) after 1985. The diagnosis of hemoglobin E was confirmed with citrate agar electrophoresis (Helena Laboratories, Beaumont, Texas) before 1985 and with the Corning Electrophoresis System after 1985. New 1% methylene blue was used to detect hemoglobin H inclusion bodies after in-vitro incubation for 1 hour at 37°C.

The serum iron level and total iron binding capacity were determined with the Ferrochem II serum Fe/TIBC analyser (ESA, Inc., Bedford, Mass.). The serum ferritin level was measured with a radioimmunoassay (Quantimune Ferritin Immunoradiometric Assay, Bio-Rad Clinical Division, Richmond, Calif.). Iron deficiency was defined as a transferrin saturation of less than 16% and a serum ferritin level of less than 10 μg/L.

One should use percentile curves based on a population of healthy oriental children to define anemia and microcytosis. Unfortunately, such curves are not available, so the percentile curves for hemoglobin level and erythrocyte volume developed by Dallman and Simes[8] were used. Although derived from a population of nonindigent white children, these curves may be considered comparable since in an earlier study Dallman and colleagues had found no difference in hemoglobin levels between white and oriental children.[9]

Results

Only one case of hemoglobin E was diagnosed between 1975 and 1978. Nineteen cases were diagnosed in 1979–83 and 22 in 1984–86.

Of the 42 children (23 boys and 19 girls with a mean age at diagnosis of 4.3 years) 32 were from Kampuchea, 5 from Laos, 2 from Vietnam, 2 from India and 1 from Thailand. Most (33) were heterozygous (having hemoglobin AE [hemoglobin E trait]), but 6 were homozygous (having hemoglobin EE) and 3 were doubly heterozygous (having hemoglobin E–β-thalassemia).

The children with hemoglobin AE or hemoglobin EE were asymptomatic. Their physical examination gave unremarkable results, and hepatosplenomegaly was not noted in any child. The diagnosis of hemoglobin E in these children had been suspected when a "routine" complete blood count revealed microcytosis, hypochromia and target cells. The diagnosis was confirmed by means of hemoglobin electrophoresis. In contrast, the patients with hemoglobin E–β-thalassemia were symptomatic, presenting at around age 2 years with pallor and splenomegaly.

The 33 children with hemoglobin E trait generally had low-normal hemoglobin levels for their age (110 to 130 g/L) (Fig. 1). Of the 29 whose iron status we were able to evaluate, 18 (62%) had coexisting iron deficiency (Fig. 1). Of the 19 with hemoglobin levels below the third percentile for age, 16 (84%) were iron deficient. Iron deficiency was far more common in the younger patients, particularly those less than 2 years old: 12 of the 14 (86%) in that age group had concurrent iron deficiency, with hemoglobin levels much lower than expected (60 to 100 g/L). The mean hemoglobin level of the iron-sufficient children was 119 g/L, compared with 94 g/L for those with iron deficiency.

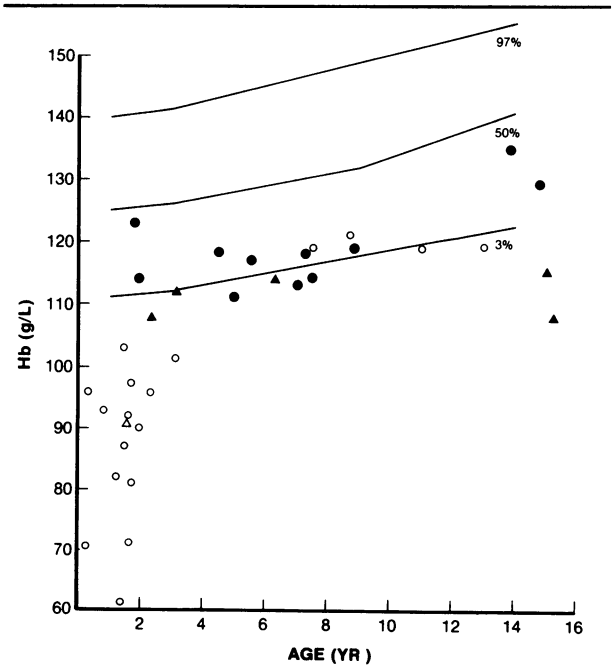

Fig. 1 — Hemoglobin level at time of diagnosis in 29 children with hemoglobin AE (○ = those with iron deficiency; ● = those without) and 6 children with hemoglobin EE (△ = those with iron deficiency; ▲ = those without). Percentile curves taken from Dallman et al.[8]

The iron-sufficient children with hemoglobin E trait generally had mean corpuscular volumes near the third percentile for age, with a mean of 74.9 fl (Fig. 2). The iron-deficient children, as expected, had much lower volumes, with a mean of only 60.4 fl.

In children with hemoglobin E trait, hemoglobin E + A_2 is expected to constitute 27% to 35% of the hemoglobin mass on electrophoresis, the remainder being mostly hemoglobin A; the proportion of hemoglobin F is less than 2% to 3%.[2,5,10,11] Of the 11 iron-sufficient patients with hemoglobin E trait, 9 had such hemoglobin E levels. In contrast, 14 of 15 iron-deficient children had hemoglobin E levels lower than expected. The results for three iron-deficient infants less than 9 months old are not included, as the change from fetal to adult hemoglobin was probably not complete. Testing for hemoglobin H inclusion bodies gave negative results in all the patients.

In two iron-deficient children with hemoglobin E trait the hematologic tests were repeated after oral iron therapy. Their hemoglobin levels increased from 71 to 107 g/L and from 93 to 114 g/L, their mean corpuscular volumes from 46 to 77 fl and from 66 to 75 fl, and their proportion of hemoglobin E + A_2 from 24.7% to 29.7% and from 23.5% to 29%.

Five of the six patients with hemoglobin EE were only slightly anemic, with hemoglobin levels just under the third percentile for age (105 to 115 [mean 112] g/L) (Fig. 1). The one patient with a hemoglobin level lower than expected (91 g/L) was less than 2 years old and was the only patient in this group with underlying iron deficiency. The five iron-sufficient children had disproportionately low mean corpuscular volumes for their degree of anemia, with a mean of 57.6 fl (Fig. 2). Hemoglobin electrophoresis revealed that the proportion of hemoglobin E + A_2 in all six patients was 93% to 99%, the remainder being hemoglobin F. Testing for hemoglobin H inclusion bodies gave negative results in all the patients.

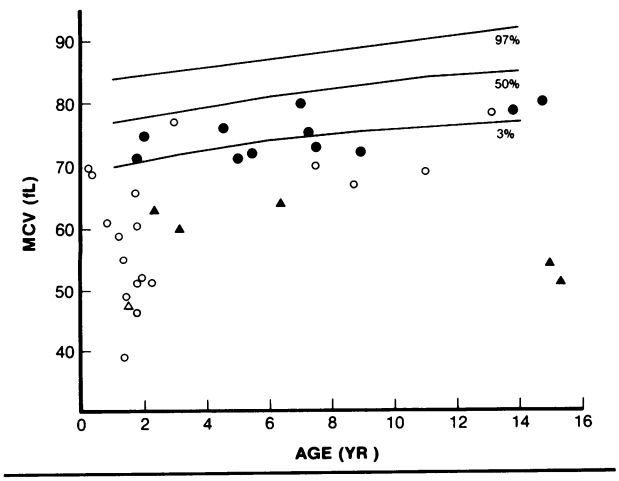

Fig. 2 — Mean corpuscular volume (MCV) at time of diagnosis in the 35 children. Symbols as in Fig. 1. Percentile curves taken from Dallman et al.[8]

The three patients with hemoglobin E-β-thalassemia had marked anemia, with hemoglobin levels ranging from 60 to 80 g/L. Their blood films revealed marked hypochromia, microcytosis, target cells, teardrop cells, anisopoikilocytosis and nucleated erythrocytes. The hematologic findings in these patients are shown in Table I.

Discussion

Children with isolated hemoglobin E trait are asymptomatic and have normal or slightly decreased hemoglobin levels.[10,12] However, the high prevalence of concurrent iron deficiency in these children may change their clinical and hematologic presentation. This is illustrated by the normal or near-normal hemoglobin levels in our iron-sufficient patients with hemoglobin E trait, as opposed to the mild to moderate anemia in those with iron deficiency. Therefore, the finding of significant anemia in children with hemoglobin E trait warrants further investigation to rule out underlying iron deficiency.

Hurst and colleagues[12] reported that almost all of their pediatric patients with hemoglobin E trait had low mean corpuscular volumes. We found that volumes lower than the third percentile for age were usually the result of iron depletion and that iron-sufficient children usually had normal volumes. Alpha-thalassemia trait may also result in values lower than expected and should be suspected when the hemoglobin level and mean corpuscular volume do not increase with iron therapy. The prevalence rate of α-thalassemia genes in Southeast Asian populations is estimated to be as high as 30%.[13] Testing for hemoglobin H inclusion bodies gave negative results in all our patients. This test, however, may not detect α-thalassemia-2 and α-thalassemia-1 doubly heterozygous states.[14] Globin-chain synthesis or gene-mapping studies may therefore be necessary to rule out asymptomatic carriage of α-thalassemia genes.

Homozygosity for the hemoglobin E gene, in

Table I — Hematologic findings in three patients with hemoglobin (Hb) E-β-thalassemia

Finding	Patient no. 1	2	3
Age, yr	2	5	16
Hb level, g/L	82	69	63
Erythrocyte count, X 10^{12}/L	4.5	2.9	3.8
Reticulocyte count, %	3	4	10
No. of nucleated erythrocytes per 100 leukocytes	–	12	531
Mean corpuscular volume, fl	61	75	74
Mean corpuscular Hb, pg	18	24	17
Hb E + A_2, %	38	28	72
Hb F, %	62	72	28
Ratio of α to non-α chains	1.4	–	–

contrast to other hemoglobinopathies (e.g., hemoglobin SS and hemoglobin CC), does not result in illness.[5,15] These patients have only mild anemia, with a moderately low mean corpuscular volume, as was the case with our patients. Since complete blood counts and blood films of homozygotes are usually similar to those of heterozygotes, a definite diagnosis of hemoglobin EE can be made only by means of hemoglobin electrophoresis, which would show more than 90% hemoglobin E + A_2, the remainder being hemoglobin F.[5]

Tittle and colleagues[16] reported that 28% of Indochinese children living in California were iron deficient. We found that 54% of our patients with hemoglobin AE or hemoglobin EE in whom iron studies were done had underlying iron deficiency. This difference may be attributed to the larger number of younger patients in our study (mean age 4.3 years, compared with 7 years in the study by Tittle and colleagues). In our study iron deficiency was far more prevalent in the children less than 2 years of age (87%) than in those aged 2 or older (30%).

Nutritional factors are by far the main cause of iron deficiency during the first 2 years of life. However, in addition to a detailed dietary history, these patients should be screened for parasites that may cause chronic blood loss. Up to 37% of newly immigrated Indochinese children may harbour hookworm or *Trichuris* in their gastrointestinal tract.[16,17] These parasites were present in seven of the nine patients that we screened.

The β-thalassemia gene is also relatively prevalent in Southeast Asian populations; the rate is estimated to be as high as 5%.[18] The doubly heterozygous state of hemoglobin E-β-thalassemia results in chronic hemolytic anemia, with a clinical picture similar to that in β-thalassemia intermedia or major.[19] Two of our doubly heterozygous patients are currently on a transfusion program. The blood films in this state resemble those in other thalassemia syndromes. However, the diagnosis is easily confirmed with hemoglobin electrophoresis, which will show 25% to 85% hemoglobin E + A_2, the remainder being hemoglobin F.[19,20] Hemoglobin A will be detected only if the patient has hemoglobin E-β^+-thalassemia.[20]

Conclusion

As a consequence of the recent immigration of Southeast Asians, hemoglobin AE and hemoglobin EE are becoming increasingly frequent diagnoses in Canada. They are benign conditions characterized by low-normal hemoglobin levels and mild microcytosis, and they can readily be diagnosed with hemoglobin electrophoresis. Coexisiting iron deficiency is common, especially in children under the age of 2 years, and should be suspected when the hemoglobin level and mean corpuscular volume are lower than expected. In addition, an unexpectedly low proportion of hemoglobin E in heterozygotes is suggestive of superimposed iron deficiency or coexisting α-thalassemia trait. Since children with hemoglobin E-β-thalassemia have moderately severe anemia and require frequent transfusion, genetic counselling for parents carrying both these genes is indicated.

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