Hemoglobin E Trait Reexamined: A Cause of Microcytosis and Erythrocytosis

By Virgil F. Fairbanks, Gerald S. Gilchrist, Bernadine Brimhall, John A. Jereb, and Edgar C. Goldston

The current Indochinese resettlement program in the United States has resulted in an increase in the number of persons with hemoglobin E trait. American physicians should be aware of the hematologic expressions of this innocuous condition. The hematologic manifestations of 21 persons with hemoglobin E trait were evaluated. The subjects were of Tai-dam, Vietnamese, Chinese, Laotian, and European origin. These studies showed uniform hematologic manifestations in hemoglobin E trait, characterized by slight microcytosis, by morphologic features resembling those of thalassemia minor, and often by increased erythrocyte count. Hemoglobin instability also was confirmed.

Although hemoglobin E (HbE) is one of the most prevalent hemoglobinopathies of man, it has been virtually limited to Southeast Asia, where altogether it may be estimated to occur in about 30,000,000 people. Only rarely had HbE been encountered in North America until the resettlement of large numbers of Southeast Asians within the United States. The hematologic features that we have observed in HbE trait contradict earlier observations. Microcytosis and, frequently, mild erythrocytosis are characteristic of HbE trait. These features must be recognized in order that persons with this innocuous condition not be subjected to unnecessary medical examinations and inappropriate treatment.

MATERIALS AND METHODS

Twenty-one persons with HbE trait were studied. They represented seven unrelated kindreds of Tai-dam, Vietnamese, Chinese, Laotian, and European ancestry. Also studied for statistical comparison were all available first-degree relatives (15 parents, siblings, or children) whose hemoglobin genotypes by electrophoresis were AA. Blood specimens were anticoagulated with EDTA sodium, and hematologic data were obtained with an automated cell counter (Coulter model S). (As indicated in Table 1, many of these data were obtained on mailed-in specimens approximately 24 hr after venipuncture.) Electrophoresis of hemolysates was performed at pH 8.6 and at pH 6.2. Serum iron concentration, total iron-binding capacity, and serum ferritin concentration were measured in some cases. Hemoglobin F was measured as alkali-resistant hemoglobin. Hemoglobin fractions were estimated densitometrically from cellulose acetate membranes stained with Ponceau-S. Hemoglobin samples from cases 10 and 19 were fractionated. Tryptic peptides were separated by column chromatography, and an abnormal peptide (218–26) was identified by amino acid analysis as containing the substitution characteristic of hemoglobin E. Statistical analyses were by Fisher's exact method, by t test, and by χ² test.

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RESULTS

Persons with HbE trait generally had normal values for hemoglobin concentration. Half of them had mild microcytosis, and all had microcytosis (Tables 1 and 2). Blood films typically showed microcytosis, poikilocytosis, and target erythrocytes. Relative concentration (proportion) of HbE was 19%–34% (median 30%) of total Hb. Hemoglobin instability was consistently shown by heat, by 17% isopropanol, and by incubation with 2,6-dichloroindophenol as previously reported.13,14

Among adult (>15 yr old) first-degree relatives who were examined, nine were found by electrophoresis to be of hemoglobin genotype AA; none had microcytosis. Five of these persons were of Tai-dam and four of European ancestry. Six Tai-dam children (siblings or progeny of cases in Table 1) who were genotypically HbAA had MCV values of 74–87 fl, conforming to the normal range for age-matched whites.15

We compared MCV values in mailed versus freshly drawn blood specimens from cases. Hematologic data were obtained approximately 24 hr after the blood was received in the mail. Comparison of MCV values in mailed versus freshly drawn blood specimens indicates no significant difference (p = 0.3) when stratified according to patient’s age.

### Table 1. Blood Values for Persons With Hemoglobin E Trait

<table>
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<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Ancestry</th>
<th>Hb (g/dl)</th>
<th>RBC (x 10^12/liter)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>HbE (%)</th>
<th>HbF (%)</th>
<th>Ferritin (ng/ml)</th>
<th>Serum Iron (TIBC) (µg/dl)</th>
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*Blood specimens from these persons were received in the mail. Hematologic data were obtained approximately 24 hr after the blood was drawn. In other studies, we showed a slight increase of approximately 2% in MCV values during 24-hr storage of EDTA-anticoagulated blood at room temperature. In cases 2 and 10, we examined both freshly drawn and mailed specimens (obtained on different occasions). MCV values were 73 and 71 fl, respectively, in case 2 and 74 and 72 fl, respectively, in case 10. A comparison of MCV values in mailed versus freshly drawn blood specimens in the series indicated mean values of 70.3 and 70.8 fl, respectively (p < 0.05 for the null hypothesis). MCH, hemoglobin concentration, and erythrocyte count did not change during more than 72 hr of storage in EDTA anticoagulant. Comparison of HbE proportion in fresh versus mailed specimens indicates no significant difference (p = 0.31, Wilcoxon rank sum test, two-tailed) when stratified according to patient’s age.
In Southeast Asia, HbE trait, α-thalassemia minor, and iron deficiency all are believed to have a high prevalence. Concurrence of either iron deficiency or α-thalassemia with HbE trait is known to result in lower proportion of HbE. Because of this and because of a possible bimodality of the proportion of HbE in our series, we reexamined the association of HbE and microcytosis after exclusion of cases with HbE in proportions of less than 29% of total Hb. There was a highly significant association \( p = 6 \times 10^{-8} \) between microcytosis and HbE trait in the 15 cases with HbE proportions of 29%-34%. Although these considerations do not prove a cause and effect relationship between HbE trait and microcytosis, they indicate a vanishingly small likelihood that the association between HbE trait and microcytosis is either coincidental or due to concurrence of other causes of microcytosis, such as thalassemia or iron deficiency.

### DISCUSSION

In 1956, Chernoff et al.\(^7\) presented data on 36 subjects who had HbE trait, with an extraordinary range in MCV values (Fig. 1). They believed that many of the Thai subjects of their study may have been iron deficient (although they had no
other evidence of iron deficiency), and they concluded that "... hemoglobin E trait is unassociated with ... hematologic abnormality." This conclusion has been widely accepted.

Subsequently, a few instances of HbE trait in North America have been accompanied by microcytosis in some cases and normocytosis in others, but there have been too few cases to allow meaningful conclusions. Only the data of Chernoff et al.\(^7\) permit comparison with ours with respect to erythrocyte size. The manual method that they employed presumably gave normal ranges corresponding to those of Wintrobe.\(^8\) In Fig. 1 the normal distributions found by Wintrobe are compared with the data of Chernoff et al. For the latter, approximately 50% of the values were microcytic. The findings of Chernoff et al. may reflect only the inherent imprecision of the manually determined MCV.\(^19-21\)

Keller and Kohne\(^22\) studied a German family in which nine members had HbE trait; all nine showed microcytosis. The hematologic expression of HbE trait appears to be unconditioned by geographic or ethnic origin.

Iron deficiency is believed to be common in Southeast Asia and previously has been invoked as the explanation of the microcytosis seen in HbE trait in Thais. However, in this series, all five of the adults tested and both of the children tested had normal iron concentrations, and all three who were tested for serum ferritin concentration had normal values. Wasi et al.\(^23\) found that in AE heterozygotes with concomitant iron deficiency the proportion of HbE was reduced to 14%-26%; even after iron therapy the proportion of HbE was only 21%-28%. In our cases, the proportion of HbE was even higher than in their iron-repleted heterozygotes. The proportions of HbE in our cases were compared with those reported in other series (Fig. 2A). Higher proportions of HbE have been observed in only two series, both from India: these may reflect methodologic differences.

Alpha-thalassemias are highly prevalent in Southeast Asia. Therefore the concurrence of HbE trait and microcytosis in these cases might be considered to represent inheritance of both the gene for HbE and one for \(\alpha\)-thalassemia. Our patient 1 (Table I) was a Tai-dam male with HbE trait and microcytosis; his wife had only HbA and was hematologically normal. If patient 1 had an \(\alpha\)-thalassemia gene, this would assort independently from the HbE gene among his children. Hemoglobin E trait and microcytosis would then be randomly distributed. He had 11 children, of whom 6 (Table 1, cases 2–6 and 14) had HbE trait and microcytosis and 5 had both HbA and normal erythrocyte size. The highly significant \((p = 0.002)\) association between HbE trait and microcytosis in these 11 siblings allows only the remotest possibility that \(\alpha\)-thalassemia was responsible for the microcytosis accompanying HbE trait.

Tuchinda et al.\(^28\) analyzed the distribution of HbE proportions in five families of Thai origin whose members had HbE trait together with various thalassemias (Figs. 2B and 2C). The proportions of HbE in five simple HbE heterozygotes were of 30%–33.1%. Our 21 subjects were compared with their 5 simple AE heterozygotes. There was no significant difference overall in proportions of HbE \((p \approx 0.3)\). Of our cases, 71% had proportions of HbE corresponding to those of their simple AE heterozygotes. We cannot exclude the possibility that 6 of our cases with HbE proportions of 19%–27% may have been doubly heterozygous for HbE and an \(\alpha\)-thalassemia gene. However, young children \((<10\text{ yr of age})\) more often had
**HEMOGLOBIN E TRAIT**

<table>
<thead>
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<th>Hb genotype</th>
<th>Cases</th>
<th>Ref.</th>
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</tr>
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<td>Present series</td>
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</tbody>
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**Diagram A**

- Simple AE
- Hb genotype
- Cases
- Ref.

**Diagram B**

- AE, Fe def. before Rx
- E/α thal, α thal
- Simple AE
- E/α thal, α thal
- E/α thal
- E/α thal

**Diagram C**

- Distribution (all cases)
- Distribution (all cases)
- Hb E conc. (% of total Hb)

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**Fig. 2.** Comparison of HbE proportions reported in HbE trait, expressed as percentage of total Hb and, in each case, assumed to represent 100 (HbE + HbA2) % total Hb. Horizontal lines, observed ranges; vertical bars, mean values for each study; m, median in Feldman and Rieder study. (A) Proportions of HbE found in persons believed to be simple HbE heterozygotes by the investigators who reported these studies. In addition to the data here represented, the authors are aware of data in five other reported cases of HbE trait, one a single instance and the other four being members of a kindred of Turkish nationality. In the first of these, HbE was 30.5% of total Hb. In the second, HbE proportions were 25%-32% (mean 28.8%). These values correspond closely to the authors' experience. No values have been reported above 33.5%, except in two studies from India; the higher values observed in India are unexplained. (B) Results of studies of concomitant HbE trait and iron deficiency and of concomitant HbE trait and α-thalassemias. These combinations result in lower proportions of HbE. (C) Comparison of data of Tuchinda et al. with those of the present study. Tuchinda et al. observed four modes of Hb proportion, which they related to the effect of the number of α-thalassemia genes. An age effect also may be expressed by these data. (Closed circles, data in persons ≥ 10 yr old; open circles, those < 10 yr old. Results in the present 21 cases do not differ significantly from those found by Tuchinda et al. in their 5 uncomplicated cases of HbE trait.)
lower values for HbE than did adults or older children. The same feature may be seen in the data of Tuchinda et al.28 (In Fig. 2C, patients <10 yr old are represented by open circles. For the combined data, the association was significant \[p < 0.01\].) Thus some of the low values in both series may have been age related. Nonetheless, the hematologic manifestations of the 6 cases in this subgroup were indistinguishable from those of the other 15 cases.

These analyses confirm that slight microcytosis is characteristic of uncomplicated HbE trait. We have no satisfactory explanation for the occasionally reported isolated instances of HbE trait with normocytosis.

As a result of the Indochinese resettlement program, HbE trait will be encountered in the United States more frequently as a cause of otherwise unexplained microcytosis, “thalassemic blood picture,” or erythrocytosis. Recognition of the hematologic expression of this innocuous condition will obviate needless and expensive medical examinations.

ACKNOWLEDGMENT

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Since this manuscript was accepted for publication, we have encountered seven additional instances of hemoglobin E trait, or 28 cases altogether in 11 unrelated kindreds. Of these 7, 1 was a woman of northern European ancestry, 2 were males of Thai ancestry, age > 15 yr, and 4 were children of Thai ancestry. The 3 adults had MCV values ranging from 70 to 77 fl (mean 73), MCH ranging from 22 to 25 pg, erythrocyte counts ranging from 5.21 to 6.44 X 10^12 per liter, and hemoglobin E proportions ranging from 32% to 36% (mean 34%) of total hemoglobin. The 4 children had MCV values ranging from 66 to 74 fl (mean 70 fl), MCH values ranging from 22 to 24 pg, erythrocyte counts ranging from 4.65 to 5.87 X 10^12 per liter (mean 5.30 X 10^12), and hemoglobin E proportions ranging from 31% to 35% (mean 33%) of total hemoglobin. These results are essentially the same as in the earlier 21 cases, although 3 of the 7 cases had hemoglobin E proportions minimally greater than 34%.

In the entire series of 28 cases, the stated proportion of hemoglobin E is presumed to include hemoglobin A2, which migrates electrophoretically in a position identical with E. Thus, the true hemoglobin E proportion is slightly less, by about 3% (that is, a stated hemoglobin E proportion of 33% may be presumed to represent approximately 30% hemoglobin E and 3% hemoglobin A2).
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