Hematologic Findings in Southeast Asian Immigrants with Particular Reference to Hemoglobin E*†‡

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ABSTRACT

Recent immigrants from Southeast Asia were screened for hematologic abnormalities using a multichannel cell counter (Coulter S), peripheral smear, free erythrocyte protoporphyrin (FEP), isoelectric focusing, and a qualitative screen for glucose-6-phosphate dehydrogenase deficiency. Hematologic abnormalities were further defined by hemoglobin electrophoresis, globin electrophoresis, HbA₂ levels, and HbF levels. Of the 189 adults studied, 68 (36 percent) were hematologically abnormal, including 28 hemoglobin E (HbE) heterozygotes, six HbE homozygotes, 14 with α-thalassemia minor, and 10 with presumptive iron deficiency. Of the 54 people with microcytic (MCV < 80fl) red blood cells (RBC), 52 had evidence of HbE or thalassemia and two had iron deficiency alone; five had both iron deficiency and a hemoglobinopathy. Homozygosity for HbE results in an asymptomatic condition similar to thalassemia minor with microcytic RBC, large numbers of target cells, normal or slightly reduced hematocrit and >90 percent HbE. People heterozygous for HbE are asymptomatic and have hematologic findings similar to thalassemia minor with slightly reduced or low normal MCV and 25 to 35 percent HbE.

* Supported in part by the National Heart, Lung, and Blood Institute Short Term Research Training Grant #HL07491.  
† Support in part by the Bureau of Medicine and Surgery Clinical Investigation Program, Project #0-16-1297.  
‡ The opinions or assertions expressed in this paper are those of the authors and are not to be construed as official or as necessarily reflecting the views of the Department of the Navy or the naval service at large.
Introduction

Hematologic abnormalities are common in people of Southeast (SE) Asian origin. Frequently encountered conditions are the thalassemias and hemoglobin E (HbE). The possible survival advantage of people with thalassemia, HbE, or glucose-6-phosphate dehydrogenase (G-GPD) deficiency in areas of endemic falciparum malaria has been discussed elsewhere. Most SE Asians with HbE or thalassemia syndromes are asymptomatic and require no treatment; however, they often have microcytic red blood cells (RBC) and in the United States may be misdiagnosed as iron deficient.

The α-thalassemia syndromes are characterized by decreased synthesis of the α-globin chain of hemoglobin and occur with frequencies of up to 20 to 40 percent in certain areas of SE Asia. Most of the α-thalassemia syndromes occurring in SE Asians are attributed to deletion of one or more of the four α-genes normally occurring on chromosome 16. The α-thalassemia syndromes are characterized by decreased synthesis of the β-chain of hemoglobin and occur with frequencies of up to 10 to 15 percent in SE Asia. The common β-thalassemia gene in SE Asia, β°, produces no detectable β-chains.

Hemoglobin E is a β-chain variant (β Glu → Lys) which was first described in 1954. Hemoglobin E is common in people originating from SE Asia or parts of India but occurs only sporadically in other populations. Hemoglobin E is associated with the austroasiatic (Mon-Khmer) language group and occurs with frequencies as high as 20 to 50 percent, especially in areas of Thailand, Cambodia, and Laos. Both heterozygotes (HbAE) and homozygotes (HbEE), even with coexistent 1 or 2 α-gene deletions, are asymptomatic and have no apparent survival disadvantage.

Double heterozygosity for HbE and β°-thalassemia produces moderate to severe anemia.

Approximately 400,000 SE Asians have immigrated to the United States in the last few years with more than one-third settling in California. Physicians must become increasingly aware of the clinical and laboratory features of the hematologic syndromes prevalent in SE Asian populations in order to make the correct diagnosis and provide the appropriate counseling or treatment. The hematologic findings in 189 recent immigrants from SE Asia are reported, with particular reference to HbE.

Methods

Immigrants from Southeast Asia attending English classes offered by the Indochinese Education Project at San Diego City College were asked both in English and in their native language (Vietnamese, Cambodian, or Laotian) to participate in this study. This oral consent approach was approved by the Human Subjects Committee of the University of California at San Diego.

Approximately five ml of venous blood, anticoagulated with ethylenediaminetetraacetic acid (EDTA), were obtained from each of the 189 Indochinese volunteers. Peripheral smears were prepared and a hemogram was obtained on a cell counter,* calibrated with whole blood analyzed by standard methods; the microhematocrit was corrected for trapped plasma. Free erythrocyte protoporphyrin (FEP) was determined in duplicate on all blood samples using a hematofluorometer (ESA #4000). Elevated FEPs were confirmed by Piamelli's extraction method. A qualitative assay for G-6PD was performed on all samples.

* Coulter counter, model S series, Coulter Electronics, Inc., Hialeah, FL.
The component hemoglobins of all samples were analyzed by isoelectric focusing on 20 cm six percent polyacrylamide gels at 400 volts for 18 hours at 4°C and quantitated with a spectrophotometer. In addition, all samples with mean cell volume (MCV) less than 80 fl or an abnormal hemoglobin on isoelectric focusing had the following determinations: HbA2 by microcolumn chromatography, HbF by Singer alkali denaturation, and Hb electrophoresis on cellulose acetate at pH 8.6. All samples with abnormal hemoglobins were also analyzed by Hb electrophoresis on citrate agar at pH 6.0 to 6.2 and globin electrophoresis at both alkaline and acid pH.

All people with hematologic abnormalities received written reports of the findings for their medical record. Patients with iron-deficiency anemia or unexplained anemia were referred to a physician. Seventeen additional SE Asians with HbE were also studied. Ten are relatives of three of the men in the original study group of 189. Seven are patients recently seen at NRMC, San Diego.

Results

The hematologic data are summarized in tables I, II, and III. The original study population consisted of 154 men and 35 women who appeared healthy and were unrelated to each other except for four women. One hundred men and 21 women were classified as controls on the basis of essentially normal hematologic values: Hct > 0.41 in men or >0.36 in women, MCV ≥ 80 fl, FEP < 40 mcg per dl whole blood at Hct = 0.35, and normal hemoglobin electrophoresis. Two men with slightly elevated MCV and one man with slightly elevated Hct and MCV were, however, included in the controls. Hematologic abnormalities were found in 68 people (36 percent of total population), including 12 percent of Vietnamese, 21 percent of Laotian-Hmong, and 48 percent of Cambodians. A total of 80 diagnoses were made in the 68 people: HbAE (28), HbEE (6), α-thalassemia minor (14), possible α-thalassemia minor (7), β-thalassemia minor (2), probable iron deficiency (10), anemia with normal FEP (9), G-6PD deficiency (3), and abnormal fast hemoglobin (1). One patient had three diagnoses, 10 patients had two diagnoses, and 57 patients had one diagnosis.

The diagnosis of HbE was established in 34 people (28 heterozygotes and six homozygotes) on the basis of an isoelectric focusing band at the same level as a

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**TABLE I**

Hematologic Findings in an Immigrant Indochinese Population

<table>
<thead>
<tr>
<th></th>
<th>Viet-</th>
<th>Camb-</th>
<th>Lao-</th>
<th>Laotian-</th>
<th>Hmong</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td><strong>Total Population</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Men</td>
<td>35</td>
<td>30</td>
<td>59</td>
<td>30</td>
<td>154</td>
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<tr>
<td>Women</td>
<td>10</td>
<td>7</td>
<td>14</td>
<td>4</td>
<td>35</td>
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<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>37</td>
<td>73</td>
<td>34</td>
<td>189</td>
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<tr>
<td><strong>Controls (HbAA,† FEP‡ &lt;40, MCV§ &gt;80, Hct$ &gt;0.41 in men or &gt;0.35 in women)</strong></td>
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<tr>
<td>Men</td>
<td>30</td>
<td>13</td>
<td>31</td>
<td>26</td>
<td>100</td>
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<tr>
<td>Women</td>
<td>10</td>
<td>3</td>
<td>7</td>
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<td>21</td>
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<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>16</td>
<td>38</td>
<td>27</td>
<td>121</td>
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<tr>
<td><strong>Hemoglobin E (HbAE or HbEE)</strong></td>
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<tr>
<td>Men</td>
<td>2</td>
<td>10</td>
<td>19</td>
<td>0</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>12</td>
<td>20</td>
<td>0</td>
<td>34</td>
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<tr>
<td><strong>Thalassemia minor (14 alpha, 7 possible alpha, and 2 beta)</strong></td>
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<tr>
<td>Men</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>4</td>
<td>14</td>
<td>3</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><strong>Iron Deficiency (presumptive, based on elevated FEP)</strong></td>
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<td></td>
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<tr>
<td>Men</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*Adult hemoglobin, †Mean cell volume, ‡Free erythrocyte protoporphyrin, §Hematocrit.
known HbEE control and on the basis of an electrophoretic band which migrated with HbC on alkaline Hb electrophoresis, with HbA on acid citrate agar Hb electrophoresis and, similarly, to the β-chain of HbC on both acid and alkaline globin electrophoresis. Hemoglobin E was found in 32 percent of Cambodians, 27 percent of Laotians, 4 percent of Vietnamese, and none of Laotian-Hmong.

Of the 28 heterozygotes for HbE, one man and one woman had concomitant iron deficiency and one woman had a possible concomitant 3 α-gene deletion with MCV of 53 fl, Hct of 22.8, FEP of 37, and 14 percent HbE. Hematologic findings in the 24 men with uncomplicated HbAE were as follows: MCV of 66 to 87 fl with eight having MCV ≥ 80 fl; RBC of 4.66 to 7.01 × 10⁶ per mcl, five with RBC < 5.5 and two with RBC < 5.0; Hct of 0.38–0.52, two with Hct < 0.41; HbF < 2 percent except for one person with 2.3 percent; and percent HbE ranging from 24 to 35 percent with mean of 30 percent. The person with 24 percent HbE also had the MCV of 66 fl and may have a concomitant deletion of 1 or 2 α-genes. Peripheral smear exam in 24 men with uncomplicated HbAE revealed that 18 had findings suggestive of hemoglobinopathy (target cells, microcytes, ovalocytes, hypochromic cells); the remaining six men had minimal RBC abnormalities.

Six men, three Cambodians and three Laotians, had homozygous HbE. Hematologic values for these six men and two healthy women with HbEE recently seen at the Naval Hospital, San Diego, CA are listed in table III. Peripheral smears in all eight people with HbEE showed large numbers of target cells, with microcytes and scattered ovalocytes.

Fifteen additional SE Asians with heterozygous HbE were studied. Thirteen were relatives of five of the people with
HbEE, including 11 children, one brother, and one mother. All 15 HbE heterozygotes had low normal or reduced MCV, low normal or slightly reduced Hct, normal FEP, and 26 to 35 percent HbE. Ten of the 15 had RBC morphology suggesting hemoglobinopathy (primarily targets and microcytes); the remainder had minimal RBC abnormalities.

A presumptive diagnosis of α-thalassemia minor was made on 11 men and three women on the basis of MCV < 76 fl, elevated or high normal RBC count, normal FEP, normal HbA₂ and HbF, and normal Hb electrophoresis. The RBC count was >6.0 in all but one man and >5.3 in the three women. There was a range from 62 to 75 fl for MCV, and Hct was below normal in only one of the 14 people. All 14 people had blood smears typical of thalassemia minor with microcytes, ovalocytes, hypochromia, and scattered target cells. An additional seven people were classified as possible α-thalassemia minor on the basis of MCV of 77 to 79 fl, high normal to increased RBC count, normal FEP, normal HbA₂ and HbF, and normal Hb electrophoresis.

One man and one woman, both Laotian, were classified as β-thalassemia minor on the basis of elevated RBC count, decreased MCV (<65 fl), normal FEP, and elevated HbA₂. Peripheral smears were typical of thalassemia minor.

A diagnosis of presumptive iron deficiency was made in six men and four women on the basis of low or low normal MCV and an elevated FEP. One person had a borderline low Hct, and the remainder had mild anemia. Three men had a disproportionately low MCV and probably had concomitant thalassemia minor. One man and one woman had concomitant HbAE.

Nine people had anemia with a normal FEP. Three had HbEE, two had HbAE, one had β-thalassemia minor, one had α-thalassemia minor, and two had HbAA.

The anemia was of mild degree except in one woman with 14 percent HbE (see previous data).

Three people had G-6PD deficiency. All three were men of Laotian extraction. One had concomitant HbE, one had concomitant HbEE, and the third had no other hematologic abnormality.

One individual (of Cambodian extraction) had an abnormal hemoglobin which migrated anodal to HbA on alkaline Hb electrophoresis, with HbA on acid citrate agar Hb electrophoresis, and similar to HbJ on globin electrophoresis. The Center for Disease Control confirmed the findings and classified the abnormal hemoglobin as a β-chain variant with electrophoretic mobility similar to Hb New York.

Discussion

Evaluation of 189 SE Asian immigrants by clinical laboratory methods revealed a 36 percent incidence of hematologic abnormalities. Of the 68 people with hematologic abnormalities, 34 had HbE and 23 had thalassemia minor. Ten people required further evaluation for probable iron deficiency and two required further evaluation for normocytic anemia. One patient had a moderately severe microcytic anemia associated with 14 percent HbE. Three people had G-6PD deficiency by qualitative screening. The reported incidence of G-6PD deficiency in SE Asia is variable, with frequencies up to 24 percent. Since the study population is small and non-random, it may not reflect the true incidence of these hematologic abnormalities in the population.

The percentage of HbE in heterozygotes is usually 25 to 35 percent, as compared to the 35 to 45 percent usually seen in heterozygotes for stable β-chain variants. The unexpectedly low percentage of HbE in heterozygotes has been attributed to both instability of HbE.
and decreased synthesis of HbE. Recent evidence suggests that the low percentage of HbE in heterozygotes is due to decreased synthesis of $\beta^E$-mRNA. Concomitant iron deficiency or $\alpha$-gene deletions may further reduce the percentage of HbE. Separation of the effects of HbE from those of $\alpha$-gene deletions is difficult by routine methods and requires family studies or sophisticated molecular studies. One study reported 24 to 30 percent HbE and microcytic RBC in a small number of HbE heterozygotes with four normal $\alpha$-genes (by deoxyribonucleic acid mapping). Despite the common occurrence of HbE in certain SE Asian populations, relatively few hematologic data are available on large numbers of HbE heterozygotes studied with modern cell counting methods. The reported values for MCV in HbE heterozygotes have varied widely. The greatest variation is in the older literature and may be due, in part, to the inaccuracies of hemacytometer RBC counts. Microhematocrit readings are also falsely elevated by trapped plasma, particularly with increased aniso- and poikilocytosis. More recent studies on relatively small numbers of people suggest that almost all people with HbAE have microcytic RBC. However, of 24 adult men and one woman with uncomplicated HbAE in the present study, 17 had a decreased MCV and eight had a low normal MCV. Since gene mapping was not performed, the possible effect of a concomitant 1 or 2 $\alpha$-gene deletion on the MCV of people with HbAE cannot be evaluated.

People heterozygous for HbE have an asymptomatic mild thalassemia-like syndrome owing to decreased production of $\beta^E$-globin. The MCV is usually slightly reduced but may be in the low normal range. The RBC count is normal or slightly increased. The hematocrit is usually normal but may be slightly reduced. The peripheral smear usually shows mild to moderate RBC abnormalities (targets, microcytes, ovalocytes) but may show only minimal RBC abnormalities. Most HbE heterozygotes are readily detectable because of microcytic RBC and abnormal blood smear. However, those HbE heterozygotes with both MCV and blood smear within normal limits will be detected only by electrophoretic screening or family studies.

People with homozygous HbE have an asymptomatic mild thalassemia-like condition characterized by microcytic RBC, numerous target cells, normal or slightly reduced hematocrit, >90 percent HbE, and <10 percent HbF. Although some older studies show normal MCVs in people who are apparently homozygous for HbE, more recent studies demonstrate that people with HbEE have microcytic RBC with numerous target cells. In the present study, the eight people with HbEE have MCVs ranging from 57 to 70 fl and all have large numbers of target cells.

Most older studies state that people with homozygous HbE may have anemia. Using a restrictive definition of HbEE which required confirmatory family studies, Fairbanks could find only seven acceptable cases of HbEE in the literature, all of which had normal or only slightly reduced hematocrits. With the present-day clinical laboratory evaluation of hemoglobinopathies, it is unlikely that such a restrictive definition of HbEE is necessary for clinical purposes. The only other known condition which could be confused with homozygous HbE is double heterozygosity for HbE and $\beta^*$-thalassemia ($\beta^E/\beta^*$). The distinction should be made readily in most patients since people with $\beta^E/\beta^*$ have moderate to severe anemia and HbF > 10 percent. In the present study, blood was available from family members of five of the eight adults with HbEE and showed confirmatory evidence of HbE.
Of the eight people with HbEE, four had normal hematocrits and four had slightly reduced hematocrits; all eight had normal FEPs. Owing to the increasing number of SE Asian immigrants and the increasingly routine use of electronic cell counters which detect microcytic RBC, physicians should be aware of the clinical and laboratory features of HbE and the thalassemia syndromes. In this study, 54 (29 percent) of SE Asian immigrants had microcytic RBC (MCV < 80 fl); 52 had evidence of HbE or thalassemia minor; two had iron deficiency alone; and five had evidence of both iron deficiency and thalassemia minor. People with HbE or thalassemia minor are asymptomatic, but they must be correctly diagnosed in order to receive appropriate counselling and avoid unnecessary treatment for iron deficiency. The diagnosis of HbE or thalassemia can usually be established by examination of blood smear, Hb electrophoresis (alkaline and acid), and determination of RBC indices, FEP, HbA2, and HbF. People from SE Asia with microcytic RBC should not receive iron therapy until a diagnosis of iron deficiency is established by tests such as FEP, saturation percent of transferrin, serum ferritin, or bone marrow iron.

References

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