HEMOGLOBIN E LEVELS IN DOUBLE HETEROZYGOTES OF HEMOGLOBIN E AND SEA-TYPE α-THALASSEMIA

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Abstract. Coinheritance of α-thalassemia and hemoglobin E (Hb E) is prevalent in Thailand, where the gene frequencies of thalassemia and hemoglobinopathies are high. Hb E carriers with, concomitant inheritance of α-thalassemia 1 are known to have a lower level of Hb E. In this study, we reviewed the Hb E levels in Hb E carriers, who either had or did not have Southeast Asian (SEA)-type α-thalassemia, in order to seek a Hb E level that may be used as a predictor for concomitant α-thalassemia carrier status. The Hb E levels as measured by microcolumn chromatography in 844 Hb E carriers detected during a prenatal screening program for severe thalassemia at Chiang Mai University Hospital were reviewed. Hb E levels ranged from 12.3-35.0% (23.3±3.1%) in 751 Hb E carriers without SEA-type α-thalassemia and from 11.6-32.0% (17.0±3.7%) in 93 concomitant Hb E and SEA-type α-thalassemia carriers. The difference was significant (p<0.01). However, the absence of SEA-type α-thalassemia could not be predicted by the higher Hb E level alone, as 3% of double heterozygotes demonstrated a level of more than 25%. Our study confirms a lower Hb E level in double heterozygotes with Hb E and SEA-type α-thalassemia. Nevertheless, the difference does not provide sufficient discriminatory power for the reliable exclusion of α-thalassemia status.

INTRODUCTION

Concomitant inheritance of α- and β-globin gene mutations is prevalent in Thailand, where the gene frequencies of thalassemia and hemoglobinopathies are high (Wasi, 1978). This implies that, for prenatal thalassemia screening, mutations of both α- and β-globin genes are to be simultaneously searched for in an individual as double heterozygotes are at risk of having offspring with more than one type of severe thalassemia.

Hemoglobin E (Hb E) is the most common hemoglobinopathy in Southeast Asia, especially along the borders of Thailand, Lao PDR and Cambodia (Flatz, 1967; Sanguansermsri et al, 1987). A compound heterozygous state with Hb E and β-thalassemia gives rise to clinical disease, with a phenotype ranging from mild anemia to severe transfusion-dependent thalassemia (Fucharoen, 2001). Therefore, prenatal screening for Hb E carriers and a fetal diagnosis program would provide an alternative for couples at risk. Hb E carriers are initially screened by the Hb E Screen or DCIP test. The diagnosis is confirmed by Hb E fractionation, which is conventionally done by microcolumn chromatography (Sanguansermsri et al, 1998, Tongsong et al, 2000).

α-Thalassemia 1 is characterized by an in cis deletion of two α-globin genes. Screening for α-thalassemia 1 carrier status is included in prenatal screening since when both parents are α-thalassemia 1 carriers, the chance of having an offspring with Hb Bart’s hydrops fetalis will be 1 in 4 for each pregnancy. An α-thalassemia 1 carrier status is usually screened for either by a low MCV (mean corpuscular volume) of less than 80 fl, or increased red blood cell osmotic resistance. Further testings by DNA-based techniques are required to provide a definite diagnosis for carriers of α-thalassemia.

The laboratory part of the prenatal thalassemia screening program at our institute is comprised of an osmotic fragility test (OFT) as a
screen for α-thalassemia 1 and β-thalassemia carriers. The OFT detects only 70% of Hb E carriers, therefore the Hb E Screen is used for screening Hb E carriers. Individuals with positive results on the OFT are further tested by microcolumn chromatography to determine the Hb A₂ and Hb E levels. They are also tested with concurrently a PCR-based test designed SEA-type α-thalassemia, which is the most common cause of α-thalassemia 1 in Thailand. Individuals with a positive Hb E Screen are further tested by microcolumn chromatography to determine the Hb E level (Tongsong et al, 2000).

Hb E makes up approximately 25-30% of total hemoglobin in heterozygotes (Fucharoen, 2001). The percentage of Hb E is influenced by various factors, such as iron status and the number of α-globin genes (Wasi et al, 1967, 1968; Huisman, 1997). In Northern Thailand, the prevalence of SEA-type α-thalassemia heterozygotes is 14% (Lemmens-Zygulska et al, 1996). The prevalence of coexisting SEA-type α-thalassemia is not uncommonly seen at 10.3% among Hb E carriers (Sanguansermsri et al, 1998). As has been shown in previous studies that double heterozygosity of Hb E and α-thalassemia results in a decrease in the quantity of Hb. We retrospectively reviewed the percentages of Hb E in Hb E carriers to compare the levels between the two groups, with and without concurrent SEA-type α-thalassemia, and to seek a specific Hb E level that may be used as a predictor for a concomitant SEA-type α-thalassemia carrier status, which may help to decrease the need for PCR tests in thalassemia prenatal screening.

RESULTS

A total of 14,156 case records were reviewed. Results from 340 cases were excluded; 305 cases lacked Hb E quantification and 35 samples were not screened for Hb E. Of the remaining 13,816 cases, 1,119 (8.1%) were defined as Hb E carriers. The results of 275 Hb E carriers were excluded because α-thalassemia status had not been determined. Of the remaining 844 cases, 751 were isolated in Hb E carriers and 93 in Hb E and SEA-type α-thalassemia carriers. The Hb E levels in both groups are shown in Table 1 and Fig 1.

The ROC curve showing the sensitivity and 1-specificity of a Hb E level in predicting a negative result for SEA-type α-thalassemia is shown in Fig 2. As calculated (data not shown), if the Hb E level is more than 21.5%, the sensitivity of having a negative result for SEA-type α-thalassemia is 73.0% and the specificity is 92.5%. When the Hb E level is more than 25%, the sensitivity of having negative result for SEA-type α-thalassemia is 28.1% and the specificity is 96.8%.

DISCUSSION

Two major factors that affect the Hb E level are coexisting α-thalassemia status and iron status (Wasi et al, 1967, 1968; Huisman, 1997). The decreased availability of α-globin chains in SEA-type α-thalassemia heterozygotes results in a decrease in the rate of α- and β-globin assembly and, therefore, a decrease in hemoglobin production (Huisman, 1997). In the case of double
heterozygotes with Hb E and SEA-type α-thalassemia, the Hb E level is known to be lower than that in isolated Hb E heterozygotes (Wong et al., 1982; Huisman 1997; Sanchaisuriya et al., 1997; Sanguansermsri et al., 1998, 1999).

Sanguansermsri et al. (1998) reviewed Hb E levels in 323 persons who had a positive Hb E screen, and reported that the Hb E levels in carriers ranged from 25-30%. Sixteen of 155 patients tested by PCR were double heterozygotes with SEA-type α-thalassemia. Hb E levels in this group of patients were lower and mostly ranged 15-20%. Huisman (1997) measured the Hb E level in 20 Hb E heterozygotes with varying types α-gene mutations. It was observed that a decrease in Hb E level was directly related to the number of deficient of α-globin genes. Wong and Ali (1982) reported similar results in four members of a Vietnamese family.

Sanchaisuriya et al. (1997) studied Hb E levels and SEA-type α-thalassemia status in 112 Hb E carriers. The SEA-type α-thalassemia gene was not detected in 34 Hb carriers whose Hb E levels were above 25% while 43 of 88 cases with Hb E levels of less than 25% carried the SEA-type α-thalassemia gene. A recent study in 202 Hb E carriers showed that levels of Hb E were related to a coinherence of α-thalassemia. The Hb E levels were inversely correlated with the number of deleted or mutated α-globin genes (Sanchaisuriya et al., 2003).

Our study shows that 11% of Hb E carriers detected at our institute had concomitant inheritance of SEA-type α-thalassemia. As in previous studies, a similar decrease in Hb E levels of double heterozygotes with Hb E and SEA-type α-thalassemia was detected. At a Hb E level of more than 25%, a concomitant SEA-type α-thalassemia carrier status could be excluded with a specificity of 96.8%. This also means that up to 3% of the putative double heterozygotes dem-

<table>
<thead>
<tr>
<th>Hb E levels (in percent)</th>
<th>Hb E carriers with SEA-type α-thalassemia (N=93)</th>
<th>Hb E carriers without SEA-type α-thalassemia (N=751)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>17.0</td>
<td>23.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>11.6</td>
<td>12.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>32.0</td>
<td>35.0</td>
</tr>
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p-value < 0.01, mean difference -6.28, 95% confidence interval -7.08 to -5.49
onstrate a Hb E level of more than 25%. This exceptional group may be a combination of real Hb E and α-thalassemia carriers, Hb E/β-thalassemia with α-thalassemia, or carriers of certain Hb variants that co-elute with Hb E, such as Hb C. We were unable to prove this because of the limitations of our retrospective study. These and other unknown factors may have influenced the Hb E levels in the heterozygous carriers.

Approximately half of Hb E carriers who did not carry SEA-type α-thalassemia had a Hb E level of less than 25%. The lower Hb E levels in this group may result from iron deficiency or other types of α-thalassemia, such as a single α-globin gene deletion, or less commonly, the Thai-type α-thalassemia 1.

Although Hb E carriers without α-thalassemia 1 are more commonly seen with higher Hb E levels, the discriminatory power of the Hb E quantification is not sufficient for the reliable exclusion of α-thalassemia status. Therefore, we strongly believe that a DNA-based method, such as the PCR, should continue to be the standard method for the diagnosis of α-thalassemia carrier status in Hb E heterozygotes.

Our study confirms, on a large sample, that Hb E levels are lower in Hb E and SEA-type α-thalassemia double heterozygotes than in isolated Hb E heterozygotes. Nevertheless, because prenatal screening and fetal diagnosis require highly accurate laboratory results, DNA-based tests for the determination of α-thalassemia status should remain the standard method for diagnosis or exclusion of α-globin gene mutations.

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