Effect of the maternal $\beta^E$-globin gene on hematologic responses to iron supplementation during pregnancy$^{1,3}$

Kanokwan Sanchaisuriya, Sapan Fucharoen, Thawalwong Ratanasiri, Pattara Sanchaisuriya, Goonnapa Fucharoen, Ekkehart Dietz, and Frank P Schelp

ABSTRACT

Background: It is customary in Southeast Asia to treat pregnant anemic women with iron supplements, but anemia in this region may be complicated by thalassemia and hemoglobinopathies, which lead to an ineffective response.

Objective: The aim was to determine whether routine iron supplementation during pregnancy in this area, which has a high prevalence of thalassemia and hemoglobinopathies, is an effective control strategy for iron deficiency anemia.

Design: A prospective study was conducted. Seventy-six pregnant women, including 43 who were heterozygous for the hemoglobin E (Hb E) gene, 20 who were heterozygous for Hb E and had $\alpha$-thalassemia, and 13 who were homozygous Hb E, as well as 77 pregnant women who had no thalassemia gene, participated in this investigation. All pregnant women received a daily dose of 120 mg elemental Fe for an average of 133.5 d. Hematologic variables and serum ferritin concentrations were measured before supplementation and after supplementation at the gestational age of 28–32 wk. Differences in hematologic variables and serum ferritin were assessed.

Results: Significant differences in hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin responses were found between the nonthalassemia group and the 3 groups with the Hb E gene after adjustment for the following baseline values: age, body mass index, duration of iron supplementation, and ferritin concentration. Significant differences in the improvements in mean corpuscular volume and mean corpuscular hemoglobin values between the 3 groups indicate a poorer response at the cellular level in the pregnant women with the Hb E gene. Further analysis showed a significant difference in the hemoglobin response only for women who were homozygous for Hb E.

Conclusion: Iron supplementation during pregnancy is not beneficial for pregnant women who are homozygous for Hb E, but a routine intervention should not cause iron overload, as judged from this short observation period. Am J Clin Nutr 2007;85:474–9.

KEY WORDS: Hemoglobin E, iron supplementation, thalassemia, serum ferritin, pregnancy, iron deficiency anemia

INTRODUCTION

Iron deficiency is a common cause of anemia in pregnancy. It is standard obstetrical practice to provide iron supplementation to all pregnant women to prevent a decrease in hemoglobin concentration and a decline in iron stores (1–3). This is especially necessary in areas where women are particularly prone to iron deficiency anemia, such as in rural areas of Southeast Asia. However, anemia is also common in persons with thalassemia and hemoglobinopathies, which are prevalent in the region (4). Among these genetic disorders, hemoglobin E (Hb E) is the most common (5). This hemoglobin variant is caused by a single nucleotide base substitution at codon 26 of the $\beta$-globin gene (GAG→AAG), leading to a replacement of lysine for glutamic acid. This mutation also activates a new cryptic splice site, resulting in reduction of $\beta^E$ mRNA synthesis, which results in a $\beta^-\text{-thalassemia}$ phenotype (6, 7). Most Hb E carriers are asymptomatic, but persons who are homozygous for the gene usually have hypochromic microcytic anemia (4).

Although both iron deficiency and thalassemia are major causes of microcytic anemia, which could affect hemoglobin production, the mechanisms generating anemia in the two conditions are different. In thalassemia, there is defective globin chain production by a certain globin gene mutation, whereas iron deficiency alters heme synthesis, the process requiring iron. When these two defects occur together, the hematologic response to iron supplementation may be limited. In severe cases of thalassemia, iron stores may even be overloaded (4), and treating these patients for iron deficiency anemia could lead to inadequate responses and could unnecessarily expose them to the known adverse effects of iron treatment (8). A recent study on schoolchildren in northeast Thailand has shown that most of anemia is related to hemoglobinopathy rather than iron deficiency (9).
which indicates the importance of hemoglobinopathy on the development of anemia in this population.

The aim of the present study was to investigate the effect of maternal βE-globin gene on the hematologic responses to a standard iron supplementation during pregnancy. The hematologic responses observed in pregnant women with thalassemia and Hb E were compared with those obtained from pregnant women without thalassemia and analyzed statistically. The results obtained should provide a better insight into the effect of iron supplementation for pregnant women in areas where both iron deficiency and thalassemia are prevalent and should help in improving iron supplementation programs in the regions.

SUBJECTS AND METHODS

Subjects and specimens

Ethical approval of the study protocol was obtained from the ethical committees of Khon Kaen University, Khon Kaen, Thailand (HE 451131). Informed consent was obtained from all participating persons. Pregnant women with their first pregnancy who attended the antenatal care service for the first time were asked to participate in this study. The recruitment of subjects took place consecutively from January 2003 to June 2004 at the Chumpea and Nampong district hospitals in Khon Kaen province, Northeast Thailand. Physical examination was conducted by well-trained and experienced nurses to determine gestational age, weight, height and blood pressure. Only apparently healthy pregnant women with gestational age of <20 wk based on last menstrual period (LMP) were recruited. Pregnant women with multiparity, currently on iron treatment, or with chronic inflammatory diseases or malaria were excluded. All women received the same formula and daily dosage of 120 mg elemental Fe supplementation without folic acid. The women’s iron intake compliance was assessed through interviews and pill-count conducted during their monthly visits. Only those women who took pills daily and with a pill count left <5 tablets/mo were retained for the study. Pregnant women were classified into 4 groups based on hemoglobin and DNA analyses: a nonthalassemic group and 3 groups with different βE genotypes, namely heterozygous Hb E, heterozygous Hb E with α-thalassemia and homozygous Hb E. Other forms of thalassemia were excluded.

Hematologic and serum ferritin analyses

Complete blood counts were performed by using an automated analyzer (Coulter Gen S; Coulter Electronics, Hialeah, FL), which is routinely run in our laboratory. Hemoglobin patterns and quantifications were measured by using an automated HPLC system (Variant; Bio-Rad Laboratories, Hercules, CA). The following hematologic variables were assessed: red blood count (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and RBC distribution width (RDW). Serum ferritin concentrations were measured in duplicate after separation of serum from clotted blood samples by using an enzyme-linked immunosorbent assay (ELISA) commercial kit (General Biologicals Corp, Hsin-Chu, Taiwan). A commercial control serum with a ferritin concentration of 50 ng/mL was used to monitor the assay performance. CVs of 6.5% and 9.8% were obtained from intra- and inter-assay reproducibility, respectively. A hemoglobin value <11 g/dL was considered as anemia, and iron deficiency (ID) was defined by a serum ferritin concentration <20 ng/mL (1–4).

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes by using a standard procedure. The βE-globin genotype was determined by an allele-specific polymerase chain reaction (PCR), as described previously (10). Identification of α-thalassemia, α+-thalassemia (3.7 and 4.2 kb deletions), hemoglobin Constant Spring (Hb CS), hemoglobin Pakse’ (Hb Ps), and common β-thalassemia genes in Thailand were performed routinely in our laboratory by using PCR methodologies described elsewhere (11–17).

Statistical analysis

The data did not follow a normal distribution; therefore, non-parametric statistical methods were applied for the description of the data and the analytic assessment. For the calculation of the medians and the 95% achieved CIs, as well as for performing other nonparametrical tests, the Minitab software (1998; release 12.2; Minitab Inc, State College, PA) was used. For model building, the multivariate median regression was applied with the use of STATA version 8 program (Stata Corp, College Station, TX). These analyses included descriptive statistics on hematologic variables and serum ferritin concentrations at the gestational ages of <20 wk (before iron supplementation) and at 28–32 wk (after iron supplementation). A P value of < 0.05 was considered statistically significant.

To analyze the responses of hematologic parameters to iron supplementation, the difference between the values of the first and second measurements of all variables were calculated and defined as the differences in RBC (dRBC), in hemoglobin (dHb), in MCV (dMCV), in MCH (dMCH), in MCHC (dMCHC), and in ferritin (dFerritin). The Kruskal-Wallis test was applied for testing the differences between the 4 groups. Further testing with Mann-Whitney U test was done when a significant difference was found. A multivariate assessment by median regression analysis of the difference in values of hematologic variables adjusted for age, BMI, duration of iron supplementation, serum ferritin, and baseline values as obtained from the groups with the βE-globin gene compared with the nonthalassemia pregnancies were done. For model calculations, the median differences both for the variables related to the nonthalassemia group (being the reference group) and for the difference of the variables of the 3 groups with Hb E were considered. In addition to calculating the overall P value, a Bonferroni adjustment was done to control for type I error.

RESULTS

A total of 153 pregnant women met all inclusion criteria and were followed up (Figure 1). Women who were finally selected for the study were initially classified into 2 groups based on
hemoglobin and DNA analyses. Group 1 consisted of 77 pregnant women without thalassemia (β^E/β^A, αα/αα), and group 2 consisted of 76 pregnant women with the β^E-globin gene. Of the 76 pregnant women with Hb E in group 2, as many as 9 different E coinherited with homozygous thalassemia (β^E/β^A, αα/αα). 2 heterozygous Hb E coinherited with α^+-thalassemia (β^E/β^A, αα/αα) and 1 homozygous Hb E coinherited with homozygous α^+-thalassemia (β^E/β^A, αα/αα). Due to the heterogeneity of Hb E genotypes, 76 pregnant women with Hb E were subdivided into 3 groups, namely pure heterozygous Hb E (n = 43), heterozygous Hb E with α-thalassemia (n = 20), and homozygous Hb E (n = 13).

The hematologic status of the women who were not included in the investigation was also assessed initially. A total of 481 women initially participated. The number of women who were in the nonthalassemia pregnancy group was 184 (38.3%) for the pure heterozygous Hb E, 101 (22.0%) for the heterozygous Hb E with α-thalassemia, and 52 (10.8%) for the homozygous Hb E; 139 (28.9%) women were carriers of other thalassemia genes. The general characteristics at baseline of the 3 groups with Hb E as well as the nonthalassemia group (n = 77) are shown in Table 1. The variables tested were not statistically significantly different between the groups.

For all 4 groups under investigation, the numbers and proportion of pregnant women who had iron deficiency anemia before and after supplementation are given in Table 2. The number of persons within most categories were, however, too small for conducting meaningful statistical tests. In general, the proportion of women with iron deficiency anemia increased for all groups except for the homozygous Hb E group.

The hematologic variables before and after iron supplementation, including ferritin, in the groups are shown in Table 3. Significant differences in the hematologic variables, including ferritin, both before and after iron supplementation (Kruskal-Wallis test followed by Mann-Whitney U test, P < 0.001 for both; Table 3) were observed between the 4 groups of pregnant women. The only significantly different hematologic response after receiving iron supplementation (after value minus before value) was found for MCV (P < 0.001, Kruskal-Wallis test).

Further analysis with a multivariate median regression adjusted for age, BMI, duration of iron supplementation, serum ferritin, and baseline values was performed. The difference in the median change of all variables under study was used for model building in a comparison of the 3 different Hb E groups with the nonthalassemia group with adjustment for the covariates as given above. The variables that significantly contributed to model

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TABLE 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nonthalassemia group (n = 77)</th>
<th>Pure heterozygous Hb E (n = 43)</th>
<th>Heterozygous Hb E with α-thalassemia (n = 20)</th>
<th>Homozygous Hb E (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>26.0 (23.0–27.2)</td>
<td>28.0 (23.0–31.0)</td>
<td>26.0 (22.0–28.0)</td>
<td>27.0 (17.8–28.7)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>13.0 (11.0–13.0)</td>
<td>13.0 (10.2–14.0)</td>
<td>14.0 (10.0–16.0)</td>
<td>13.0 (11.7–15.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>51.0 (49.8–53.3)</td>
<td>51.0 (47.8–54.3)</td>
<td>54.0 (51.1–56.5)</td>
<td>48.6 (44.6–52.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.0 (153.0–156.0)</td>
<td>153 (150.0–155.1)</td>
<td>154.5 (152.2–157.0)</td>
<td>150.0 (147.0–158.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 (20.5–22.3)</td>
<td>22.0 (20.8–22.9)</td>
<td>22.1 (21.2–23.8)</td>
<td>20.9 (19.7–23.1)</td>
</tr>
<tr>
<td>Duration of iron supplem.</td>
<td>134 (131.0–145.0)</td>
<td>142 (124.2–156.8)</td>
<td>130 (108.0–154.3)</td>
<td>130 (118.8–145.8)</td>
</tr>
</tbody>
</table>

*All values are medians; 95% CI in parentheses. No statistically significant differences were observed between the groups (Kruskal-Wallis test).*
The 3 types of Hb E had a statistically significant effect on dMCV and dMCH, but only for homozygous Hb E did iron supplementation have a statistically significant effect on dHb ($P = 0.010$).

We have shown previously that hematologic variables of non-pregnant subjects with Hb E are significantly different from those of nonthalassemic pregnant subjects (17, 18). The finding in the present study that pregnant women with $\beta^E$-globin gene have hematologic values different from women who are not carriers of this gene (Table 3) was therefore not unexpected. This result confirms that $\beta^E$-globin gene can affect hemoglobin production and is associated to some degree with anemia in pregnancy. Because of the mechanism of globin chain production, it is possible that in women with the $\beta^E$-globin gene, the response during pregnancy to iron supplementation differs from that of pregnant women who do not have thalassemia.

As shown in Table 3, MCV values were significantly higher at baseline and even after supplementation in the nonthalassemia group than in the groups of Hb E pregnancies. RBC and hemoglobin values decreased significantly. This indicated that a physiologic response to iron supplementation in pregnancy is associated with the hemoglobin production of the cell rather than an increase in erythropoiesis.

To our knowledge, there has been only one study conducted before in the northeastern part of Thailand that compared hemoglobin response to iron supplementation in pregnancy between normal hemoglobin type (Hb AA) and heterozygous Hb E (Hb EA) (19). In this previous study, a significant difference was observed in the rise of hemoglobin concentration between pregnant women with Hb AA and Hb EA after 15 wk of iron supplementation and subjects with Hb EA did not achieve the same hemoglobin concentration as those with Hb AA. The results of our study, however, point to a hemoglobin response in the...
TABLE 4

Multivariate assessment of the effect of iron supplementation on the change in hemoglobin (dHb), mean corpuscular volume (dMCV), and mean corpuscular hemoglobin (dMCH) for groups with heterozygous Hb E heterozygous Hb E with α-thalassemia, and homozygous Hb E compared with the values derived from the group of nonthalassemia pregnancies.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Difference of median change (β coefficient)</th>
<th>95% CI</th>
<th>Overall P</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>dHb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygous Hb E</td>
<td>−0.368</td>
<td>−0.788, 0.052</td>
<td>0.255</td>
<td></td>
</tr>
<tr>
<td>Heterozygous Hb E with α-thalassemia</td>
<td>−0.450</td>
<td>−1.039, 0.139</td>
<td>0.622</td>
<td></td>
</tr>
<tr>
<td>Homozygous Hb E</td>
<td>−1.195</td>
<td>−1.941, −0.448</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>dMCV³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygous Hb E</td>
<td>−4.545</td>
<td>−6.272, −2.818</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Heterozygous Hb E with α-thalassemia</td>
<td>−4.150</td>
<td>−6.003, −2.296</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Homozygous Hb E</td>
<td>−5.527</td>
<td>−8.483, −2.572</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>dMCH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygous Hb E</td>
<td>−2.000</td>
<td>−2.619, −1.359</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Heterozygous Hb E with α-thalassemia</td>
<td>−1.484</td>
<td>−2.248, −0.781</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Homozygous Hb E</td>
<td>−2.234</td>
<td>−3.331, −1.138</td>
<td>0.012</td>
<td></td>
</tr>
</tbody>
</table>

¹ The multivariate median regression analysis was applied by adjusting for age, BMI, duration of iron supplementation, ferritin, and baseline values. n = 43 for the heterozygous HbE group, 20 for the heterozygous HbE with α-thalassemia group, and 13 for the homozygous HbE group.
² Bonferroni adjustment to avoid type 1 error.
³ Influence of ferritin, difference of median change (β coefficient) = 0.011 (95% CI: 0.002, 0.020).

The different results obtained in our study compared with the previous study (19) are possibly due to the different times of measurement and globin genotypes of the subjects. In our study, the medians for the first and second measurements were 13 and 32 wk of gestation, respectively, whereas in the former study (19) the second measurement was done at 32–36 wk of gestation and globin genotype was not determined. It has previously been found that hemoglobin concentrations decrease during pregnancy, reaching its lowest point at 16 wk of gestation, and recovery of hemoglobin concentrations is completed after the gestational age of 36 wk (20). During pregnancy, maternal blood volume increases by ∼1.5 L. The increase begins during the first trimester, is most significant in the second trimester, and plateaus during the end of the third trimester (21). Therefore, the reduction in hemoglobin concentration and number of RBCs in the second measurement observed in our study could be due to the hemodilution effect of pregnancy. Because of this hemodilution effect, the crude values measured at the particular time points should not be used for direct comparisons. As shown in Table 3, the MCV values in both nonthalassemia pregnancies and all Hb E subgroups increased at the second measurement, but the difference was higher in the nonthalassemia pregnant group (88.9 to 92.2 fL). This result indicates that, similar to the findings reported previously on heterozygous β-thalassemia (22, 23), Hb E can influence the MCV response during pregnancy.

Hemoglobin concentrations in pregnancy depend on a number of factors, including the occurrence of infectious diseases. It was not possible to follow up the health status of the women and determine, in particular, infectious diseases acquired at home. However, the women visited the antenatal care centers at the 2 district hospitals according to schedule, and it is assumed that in case they contracted any serious infections, they would have told the staff of the centers during their visits. In addition, no symptom of infections was shown by the women during the hospital visits. Other factors that may have influenced the variation in hemoglobin were the age of pregnant women, gestational age, BMI, iron deficiency, and duration of iron tablet intake (19, 24, 25).

These factors may also affect the response of hemoglobin and other hematologic variables to iron supplementation. In addition, some clinical trials on the hemoglobin response to iron supplementation indicate that baseline values have to be taken into consideration (26). Therefore, a multivariate analysis was carried out to control these potential covariates, including the baseline values for each hematologic variable. On the basis of the median regression analysis, the differences of median change for hemoglobin, MCV, and MCH in pregnant women with the β-thalassemia gene were significantly lower than those of pregnant women without the gene. The difference in MCV was also affected by serum ferritin values (Table 4). It seems likely, therefore, that the maternal β-thalassemia gene plays a role in the response of hematologic variables to iron supplementation. Although serum ferritin seems to have some effect on the difference in the MCV value, hemoglobin production in pregnant women carrying β-thalassemia is limited with a similar iron supplementation protocol to that of normal pregnancies. The women do not, however, seem to be in danger of experiencing an iron overload either because serum ferritin declined after supplementation (Table 3) and none of the women required blood transfusions during their pregnancies.

Because of the heterogeneity of thalassemia and hemoglobinopathies in Southeast Asia (17, 18, 27), interaction of Hb E with various forms of α-thalassemia was unexpectedly observed in our study sample, most of which had heterozygous Hb E coinherited with α+-thalassemia. Other interactions with less frequency included heterozygous Hb E coinherited with either α+-thalassemia, Hb CS, Hb Pakse’, or homozygous α+-thalassemia. When the 3 different forms of Hb E, ie, pure heterozygous Hb E, heterozygous Hb E coinherited with α-thalassemia, and homozygous Hb E, were analyzed, highly significant differences in the responses of MCV and MCH to iron supplementation were observed between the 3 subgroups and the nonthalassemia pregnancy group (Table 4). These results confirm that all forms of Hb E are associated with limited hemoglobin production within the cell. However, when considering the overall hemoglobin value, only homozygous Hb E responded with a significant difference (Table 4, P = 0.010) to iron supplementation compared with the nonthalassemia pregnancy group. In fact, this is not surprising because the production of β-thalassemia chain is markedly reduced in homozygous Hb E compared with heterozygous Hb E. The hemoglobin content of the cell (as indicated by MCH) is therefore lower, and this is accompanied by reduced MCV and hemoglobin concentration. Thus, it is apparent from our data that pregnant...
women with homozygous Hb E may not benefit from iron supplementation. Further comparison of the serum ferritin concentrations before and after iron supplementation in pregnant women with homozygous Hb E revealed no statistically significant difference (55.1 compared with 48.0 ng/mL, Table 3). In contrast, significant reductions in serum ferritin concentrations were observed for a group of pure heterozygous Hb E (60.0 compared with 25.6 ng/mL) and heterozygous Hb E coinherit with α-thalassemia (64.0 compared with 25.1 ng/mL). However, iron deficiency could still occur in the homozygous Hb E pregnant woman as has been encountered in one of the participants. In such a case, iron supplements should be given to improve the heme production and maintain acceptable hemoglobin concentrations. It is very important in clinical practice to check that the response to iron is adequate, and if not, to search for the cause of the inadequacy (3). We showed in the present study that the homozygous Hb E genotype may be one of the important causes of the inadequate response to iron treatment in pregnant women in Southeast Asia.

As yet we are not aware of any standard practice that recommends iron supplementation in pregnant women with homozygous Hb E. Routine iron supplementation protocols are still recommended in the area and do not pose danger to women who are Hb E carriers. Further study on more such cases are required before a more appropriate iron supplementation protocol can be developed. It is conceivable, however, that thalassemia and hemoglobinopathies could play a major role in anemia control programs of pregnancy in northeast Thailand. The study by Thurlow et al cited above (9), which found that the contribution of iron deficiency to anemia was low in northeast Thai schoolchildren and the homozygous Hb E genotype was the major predictor of hemoglobin concentration, indirectly supports our finding. Therefore, thalassemia and hemoglobinopathies should be taken into consideration in the planning of anemia control programs and iron supplementation in pregnancy to provide appropriate care and counseling to pregnant women in the regions where both hemoglobinopathies and iron deficiency are prevalent.

We thank Uthai Ukosanakam and Wichai Ussavaphark, directors of Chumpas and Nanpong district hospitals, respectively, for their cooperation in the blood collection. We also thank Bandit Thinkhamrop for statistical support and Ian Thomas for helpful comments on the manuscript.

This study was planned and implemented by KS, SF, and FPS. KS was a doctoral student in the Public Health program at Khon Kaen University, Thailand, and SF was her major advisor. KS and SF were responsible for drafting the original manuscript. SF was responsible for acquisition of funds and supervision and for analysis and interpretation of data. TR was partly responsible for acquisition of funds. SF was responsible for acquisition of funding and for analysis and interpretation of data. TR was partly involved in data analysis and interpretation. PS was the field coordinator who also participated in data analysis and interpretation. GF was a consultant for drafting the original manuscript. SF was responsible for acquisition of funding and supervision and for analysis and interpretation of data. TR was partly involved in data analysis and interpretation. ED and FPS supervised all statistical analysis of the data. All authors read this manuscript and agreed with the submission, and no authors had a personal or financial conflict of interest.

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