Thalassemia and Hemoglobinopathy Despite Normal Level of Hemoglobin Concentration and Normal Mean Corpuscular Volume

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Abstract

The small mean corpuscular volume (MCV) of red blood cell is one of the indicators for thalassemia and hemoglobinopathy screening. As other screening methods, it may have some degrees of limitation. The retrospective study was performed among 104 patients who had various kinds of thalassemia or hemoglobinopathy despite having normal hemoglobin (Hb) concentration and normal mean corpuscular volume (MCV). Every participant had MCV > 80 fL and Hb > 12 g% for females and Hb > 13 g% for males, using automated hematology analyzer. Without genotype study, 104 cases from 1,054 (9.9%) samples of Hb typing using the capillary zone electrophoresis fulfilled the inclusion criteria. There were Hb E trait of 92 (88.5%), Hb CS trait of 8 (7.7%), beta-thalassemia/Hb E of 2 (1.9%), double heterozygote of Hb E and CS of 1 (0.9%) and beta-thalassemia trait of 1 (0.9%). Their mean Hb concentration, MCV and MCH were 13.4 ± 1.2 g%, 84.7 ± 3.6 fL and 26.7 ± 1.7 pg respectively. Only 27.8% of them were shown to be positive for single tube osmotic fragility (OF) test. In the case of screening, the combination of the normal Hb concentration, the normal MCV and negative OF test do not preclude the chance of any people to harbor thalassemia or hemoglobinopathy particularly in the area with especially high prevalences of these mutations like Thailand.

Key words: Thalassemia, Hemoglobinopathy, Normal Hemoglobin Concentration, Normal Mean Corpuscular Volume
INTRODUCTION

There are many algorithms for detecting the heterozygous state of thalassemia or hemoglobinopathy, for instance, the osmotic fragility (OF) with or without the dichlorophenol indolphenol precipitation (DCIP) tests and the mean corpuscular volume. If the OF with DCIP tests were used together for alpha, beta, beta±thalassaemia or Hb E screening, the sensitivity and the specificity were 98.1-100 % and 65.4-88.4% respectively. The positive predictive value (PPV) and the negative predictive value (NPV) of such combined test were 76.0-86.9% and 98.1-100% respectively. When the red blood cell (RBC) indices of MCV < 80 fL was used as the cut point, the sensitivity and specificity were 92.9 % and 83.9 % respectively in screening for alpha-thalassemia-1 and beta-thalassemia while the PPV and NPV were 37.9% and 99.1% respectively and some Hb E traits as well as Hb Constant-Spring (CS) traits with or without Hb E trait could be missed. If the mean corpuscular hemoglobin (MCH) < 26.5 pg was used to screen alpha-thalassemia-1 and beta-thalassemia, the sensitivity, the specificity, the PPV and the NPV were 95.2%, 82.3%, 40.4 % and 99.3% respectively.

With every algorithm of screening, the small percentages of thalassemia or hemoglobinopathy can be missed and it becomes problematic in the area with the strikingly high prevalences of thalassemia and hemoglobinopathy as Thailand, i.e. 62.4 % in the northeastern part of the country. If the diagnosis of only one case of severe thalassemia is missed and allowed to be born, it will result in the great social burden and cost 6.6 million Baht for life long treatment. This paper is aimed to study thalassemia or hemoglobinopathy despite having the normal Hb concentration and normal MCV.

MATERIALS AND METHODS

This retrospective study recruited the participants who were tested for Hb typing and CBC during July 2009 - December 2011. They all were 15 years old or older. The blood samples were routinely drawn from every pregnant woman who attended the antenatal clinic for the first time, the spouses and some relatives belonging to the pregnant women who were found to have thalassemia or hemoglobinopathy, for CBC using the automated hematology analyzer and for Hb typing using the capillary zone electrophoresis.

Only who had hemoglobinopathy or thalassemia in spite of having the normal Hb concentration, ie Hb > 12 g/dL for females and > 13 g/dL for males and the normal MCV, ie > 80 fL were included. Because the definition of anemia by CDC in childbearing-aged women was different, viz. Hb level less than 11 g/dL in the first and third trimesters, less than 10.5 g/dL in the second trimester and less than 12 g/dL for nonpregnant women, and our samples included both nonpregnant and pregnant women in various trimesters, the Hb level of 12 g/dL was presumed to be the cutoff point for anemia in women with or without pregnancy for assuring that all female samples were free from anemia.

The exclusion criteria consisted of the normal participants or the patients with thalassemia or hemoglobinopathy with low Hb concentration or low MCV.

The kind of thalassemia and hemoglobinopathy including other parameters such as gender,
Hb concentration, MCV, MCH and OF test would be also collected and expressed as the percentage, the mean and the standard deviation (S.D.). The study was approved by the ethic committee of Maharat Nakhonratchasima Hospital.

RESULTS

One thousand and fifty-four participants were tested for Hb typing concurrently with CBC and only 104 (9.9 %) fulfilled the inclusion criteria. There were 52 males and 52 females. Patient characteristics were shown in table 1.

The means ± S.D. of Hb concentration and of MCV of the patients were 13.4 ±1.2 g/dL and 84.7±3.6 fl, respectively.

The kind and the number of thalassemia and hemoglobinopathy were shown in the table 2.

Of 104 participants, there were 92 Hb E traits (88.5 %), 8 Hb CS traits (7.7 %), 2 beta thalassemia/ Hb E diseases (1.9 %), 1 double heterozygote of Hb E and Hb CS (0.9%) and 1 beta thalassemia trait (0.9%).

Of 104 patients, 97 (93.3 %) were simultaneously tested for the single tube osmotic fragility (OF) test and the results were shown in the table 3.

By single tube OF test among 97 participants, only 27 (27.8 %) were shown to be positive which consisted of 23 from 87 Hb E traits (26.4 %), 2 from 7 Hb CS traits (28.6 %) and 2 from 2 beta thalassemia/Hb E diseases (100 %). One case of

<table>
<thead>
<tr>
<th>Type of thal/hemoglobinopathy</th>
<th>No (%) +ve</th>
<th>No (%) -ve</th>
<th>total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb E traits</td>
<td>23 (26.4 %)</td>
<td>64 (73.6 %)</td>
<td>87</td>
</tr>
<tr>
<td>Hb CS traits</td>
<td>2 (28.6 %)</td>
<td>5 (51.4 %)</td>
<td>7</td>
</tr>
<tr>
<td>Beta-thal/Hb E</td>
<td>2 (100.0 %)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hb E trait+Hb CS trait</td>
<td>0</td>
<td>1 (100 %)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>27 (27.8 %)</td>
<td>71 (72.2 %)</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 1: The characteristics of the patients with thalassemia or hemoglobinopathy despite normal Hb concentration and normal MCV

Table 2: The kind and the number of participants with thalassemia or hemoglobinopathy despite having normal Hb concentration and normal MCV

Table 3: The results of osmotic fragility (OF) test among the participants with various thalassemia or hemoglobinopathy
double heterozygote of Hb E and Hb CS was tested and found to be negative.

**DISCUSSION**

Among 1,054 subjects, 104 (9.9 %) were found to silently have thalassemia or hemoglobinopathy despite having the normal Hb concentration and MCV > 80 fL. To compare with the study performed in Hong Kong, 96 subjects with MCV > 80 fL were screened, 34 were found to harbour globin gene mutation, 31 had deletion of one alpha globin gene, 3 had Hb E and 1 had Hb CS mutations.

From 104 patients, Hb E trait was the most common presentation, accounting for 88.5 %. It was not unusual because the high prevalence of Hb E in the northeastern part of Thailand is 39.7% to 43.2% and additionally the Hb concentration of Hb E traits usually falls within the normal range, ie. 14.1±1.01 g/dL for males and 12.6±1.10 g/dL for females while one-third of the Hb E traits have MCV > 80 fL.

Hb CS trait is the second common in our series. And it is highly prevalent in Thailand, ranging from 5.8 % in the central part to 8.5 % in the northeastern part of the country. The Hb concentration and the MCV of the Hb CS traits always appear normal, ie 12.9±1.4 g/dL and 88±4 fL respectively. Two cases are provisionally interpreted as beta thalassemia/hemoglobin E disease because their Hb typing consist of Hb A, Hb E, Hb F and Hb A2 of 56.6 %, 26.2 %, 14.0 % and 3.2 % respectively in the first case and of 63.8 %, 21.4 %, 11.2 % and 3.8 % respectively in the second case. To interpret the pattern of various Hb like these, beta thalassemia/Hb E disease is firstly considered because it is the most common form of severe beta thalassemia in Thailand. In fact, the patients with this entity have varied degree of anemia, ranging from asymptomatic to transfusion-dependent, the Hb concentration can vary from 4.2 g/dL to 12.6 g/dL, and the minority of them (0.27 %) has Hb concentration of 12 g% or more. The cause of the variable severity of anemia is believed to be multifactorial such as the co-inheritance of alpha thalassemia or the mutation associated with increased Hb F synthesis, eg XmnI polymorphism. The MCV of the patients with beta thalassemia/Hb E can range from 47.6 fL to 80.6 fL. The percentage of Hb E may range from 17.8 % to 81.0 % whereas Hb F ranges from 3.3 % to 46.6 %, mean 25.1 %. The hematological data of our patients appear to fall within the range of those of the patients with beta thalassemia/Hb E disease however the definite diagnosis needs the DNA analysis of the patients as well as the thalassemia study of the family.

Other possible interpretations of the combination of Hb E and Hb F are the delta beta thalassemia/Hb E disease and the double heterozygosity of hereditary persistence of fetal Hb (HPFH) and Hb E. The patients of these 2 combinations possess Hb E and Hb F only, the Hb concentrations are 13.9 g% and 12.7 g% respectively, the MCVs are 69±21 and 75.7±86.0 fL respectively. But our patients are less likely to be these entities because they have not only Hb E and Hb F but also Hb A and Hb A2.

In Hb E disease, the Hb concentration ranges from 11.4±1.8 g% in pure form to 12.8 g% in the combination with the deletion of 2 from 4 of alpha globin genes, and the MCV is 70±4 fL. The percentage of Hb E should be highly close to 100 % while that of Hb F can vary from 13.4 % for...
the adult to 24.6 % for the children.

All these possible interpretations of the combination of Hb E and Hb F need the study of the genotype that had not been performed therefore the definite diagnosis for our two cases cannot be surely made.

There is one case of double heterozygote of Hb E and Hb CS. To compare with the previous study, five of seven (71.4 %) of this entity have MCV > 80 fL, the Hb level is 11.1±1.1 g%, mean MCV is 81.9 ± 2.4 fL and one of seven (14.3 %) shows positive result for OF test.

Actually beta-thalassemia trait is suspected in case of MCV < 80 fL with raised percentage of Hb A2. However some beta thalassemia genes can give rise to normal hematological picture with slightly raised Hb A2, e.g., trait of IVS2+1 G > A Hpl III mutation having Hb concentration of 12.3 g% for female and 13.7 g% for male, some traits have normal RBC indices if they are co-inherited with alpha thalassemia. Liao found that 6 of 449 pregnant women with beta thalassemia trait (1.3 %) had MCV >80 fL, range 80.3 - 83.4 fL, and all of them carried -28 (A>G) mutation. However the genes were not studied in our case, the definite genotype of beta thalassemia resulting in normal Hb concentration and normal MCV value could not be concluded.

For OF test, 27 of 97 participants (27.8 %) are shown to have positive results, consisting of 26.4 % of Hb E trait, 28.8 % of Hb CS trait, 100 % of beta thalassemia/Hb E disease and none of double heterozygote of Hb E with Hb CS, respectively, comparing to 48.4 %, 15.4 %, 91.7 % and 14.3 % of the parallel groups, of the previous study. In fact, Hb E is sensitively detected by the DCIP test but it was not performed in our study while OF test is most appropriate for screening both alpha- and beta-thalassemia with very high sensitivity.

**CONCLUSION**

From 1,054 samples of Hb typing using the capillary zone electrophoresis, 104 (9.9 %) harbored thalassemia or hemoglobinopathy despite having normal Hb concentration, i.e., Hb > 12 g% for females and >13 g% for males and normal MCV, i.e. >80 fL. They consisted of Hb E trait of 92 (88.5 %), Hb CS trait of 8 (7.7 %), beta thalassemia/Hb E of disease 2 (1.9 %), double heterozygote of Hb E and CS of 1 (0.9 %) and beta thalassemia trait of 1 (0.9 %). And only 27.8 % of them had positive result for single tube osmotic fragility (OF) test. Therefore the combination of the normal Hb concentration, the normal MCV and negative OF test do not preclude the chance of any people to harbor thalassemia or hemoglobinopathy.

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**References**


