

## Variable Severity of $\beta$ -Thalassemia/Hemoglobin E Disease - the Genetic Factors

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**ABSTRACT.** Hemoglobin E (Hb E) is prevalent in Southeast Asia. Heterozygotes give no manifestation, even homozygotes show no manifestation or only slight anemia. However, compound heterozygote with  $\beta$ -thalassemia gives anemia with variable severity. The severely affected individuals show anemia similar to homozygous  $\beta$ -thalassemia. Many factors play a role in determining the severity.  $\beta^{\text{Thal}}/\beta^{\text{E}}$  patients have increased superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. The increased GSH-Px is thought to be needed for the elimination of hydrogen peroxide produced by SOD decomposition of peroxide. The content of antioxidants vitamins E and C is reduced, whereas MDA, the final product of lipid peroxidation increases significantly. The genotype of the Xmn I polymorphism, -158 bases upstream from the transcription site of the  $\alpha$ -globin and the level of Hb F are associated with clinical severity, but the extent of the  $\beta^{\text{E}}$ -globin mRNA cryptic splicing is more associated with the severity of the manifestation than does the pattern of the Xmn I polymorphism. Like in other homozygous  $\beta$ -thalassemia, coinheritance with  $\alpha$ -thalassemia ameliorates the severity of  $\beta^{\text{Thal}}/\beta^{\text{E}}$  through the reduction of the imbalance  $\alpha$ -/ $\beta$ -chain ratio and might lead to less precipitation of  $\alpha$ -polypeptide excess in the erythrocyte membrane. [Paediatr Indones 1997;37:6-12].

### Introduction

Hemoglobin E (Hb E) is one of the most frequent hemoglobin variants in Southeast Asia populations. Although it is most prevalent in Thailand, it is also a health problem in other SEA countries, i.e., Vietnam, Cambodia, Laos, Burma, Malaysia, The Philippines, and Indonesia.<sup>1</sup>

Hb E results from a single amino acid substitution of glutamic acid to lysine due to a point mutation in codon 26 (GAG- $\rightarrow$ AAG). This mutation results in the production of abnormal hemoglobin (Hb E) and activation of cryptic splicing site resulting in further reduced  $\beta$ -globin production.<sup>2</sup> Individuals with heterozygous  $\beta^E$ -gene show no anemia or other clinical manifestations. Even, homozygotes show no clinical manifestation or have only slight anemia with microcytosis and hypochromia. However, compound heterozygous  $\beta^E$  with  $\beta^{\text{Thal}}$  ( $\beta^E/\beta^{\text{Thal}}$ ) may have a severe disease, with the most severely affected individual having anemia similar to those of Cooley's anemia.<sup>3-6</sup> About half of  $\beta^{\text{Thal}}/\beta^E$  patients have low levels of Hb, i.e, less than 7 g/dL, and the rest have more than 7 g/dL.<sup>5,7</sup>

Many factors are related to the severity of  $\beta^{\text{Thal}}/\beta^E$ . The risk of oxidant stress is claimed to increase in thalassemic red cells. Suthipark et al.<sup>8</sup> reported that there were increased superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity in  $\beta^{\text{Thal}}/\beta^E$  patients. The increased GSH-Px was thought to be needed for the elimination of hydrogen peroxide produced by SOD decomposition of peroxide. Furthermore, the content of antioxidant vitamins E and C decreased, whereas MDA, the final product of lipid peroxidation increased significantly. The significant reduced vitamin E content and the increased MDA were also shown in the previous studies either in homozygous  $\beta$ -thalassemia as well as in  $\beta^{\text{Thal}}/\beta^E$ . The genetic factors, both linked and unlinked to the  $\beta$ -globin gene complex, are essential as the determinants of the severity of  $\beta^E/\beta^{\text{Thal}}$  manifestations.

Advances in molecular biology has led us in better understanding of the role of genetic factors as determinants of the severity of the phenotype of thalassemia syndrome. In this paper the variable severity of Hb E disease related to the genetic factors will be discussed

## Globin Genes

Hemoglobin consists heme and globin, the latter consists of 2 pairs of polypeptide chains. There are three types of normal hemoglobin in extrauterine life, i.e., Hb A (HbA<sub>1</sub>/adult hemoglobin), Hb A<sub>2</sub> and Hb F (fetal hemoglobin). All of the hemoglobins have 1 pair of the same polypeptide chain ( $\alpha$ -chain), while the other pairs are  $\beta$ -chain in Hb A (therefore signed as  $\alpha_2\beta_2$ ),  $\delta$ -chain in Hb A<sub>2</sub> ( $\alpha_2\delta_2$ ) and  $\gamma$ -chain in Hb F ( $\alpha_2\gamma_2$ ). The globin chain are produced in the nucleated erythrocytes up to the reticulocyte level. The production of each type of globin is coded by a specific gene. Two  $\alpha$ -genes together with other globin genes ( $\epsilon$  and  $\zeta$ -genes, both are active only in embryonal period) are located in chromosome 16 as  $\alpha$ -cluster, while  $\beta$ ,  $\delta$  and  $\gamma$  gene ( $\beta$ -cluster) are located in chromosome 11. Deletion or point mutation results in variants of hemoglobin, or, if the abnormality results in the reduction of polypeptide synthesis (imbalance of polypeptide chains production), then resulted in thalassemias.<sup>9</sup> The reduced polypeptide synthesis can occur in any globin, therefore we have  $\alpha$ -,  $\beta$ -,  $\delta$ -,

$\gamma$ -thalassemia or the compound form (e.g.,  $\beta\delta$ -thalassemia). Point mutations are the main abnormalities in  $\beta$ -thalassemia while deletions in  $\alpha$ -thalassemia.<sup>10</sup> There are more than 150 types of  $\beta$  mutations (in the-exon, the intervening sequence IVS) or outside the structural  $\beta$ -gene resulting in various severity of  $\beta$ -thalassemia).<sup>10-13</sup> Mutation in exon 1 in codon 26 (GAG- $\rightarrow$ AAG) produces Hb E. The mutation in codon 26 of the  $\beta^E$ -globin gene can activate the cryptic splice site at codons 24-27 leading to an alternative splicing which produces no  $\beta$ -globin chain (in  $\beta^{Thal}/\beta^E$ ), whereas the normal spliced mRNA which contains the exon 1 mutation at codon 26 can produce  $\beta^E$ -globin<sup>2,4</sup> and thus results in Hb E.

### Geographic Distribution

Hb E is common in Southeast Asia populations. It is also common in India Subcontinent and South China. In Sri Lanka<sup>14</sup> and in the Eastern of Saudi Arabia.<sup>15</sup> it has also been reported. Thailand is the most prevalent country for Hb E, with the  $\beta^E$  gene carrier estimated around 30% of population.<sup>1</sup> In some isolated areas frequency of nearly 60% has been found.<sup>16</sup> There are 3250 newborn babies with  $\beta^{Thal}/\beta^E$  born per million newborn per year from 13 000 couples at risk.<sup>17</sup>

In Indonesia the  $\beta^E$  gene carrier is estimated around 4%.<sup>1</sup> Studies of Hb E in various parts of Indonesia found varied rates, ranging from 0.2% in Borneo, Celebes, and Moluccas to 20.5% in the Sunda Islands.<sup>18</sup> Hb E-gene carrier was reported to be 6% in East Java,<sup>19</sup> it is prevalent in West and East Nusa Tenggara, low in Flores and Alor,<sup>20</sup> and zero among Ambonese.<sup>21</sup> The different rates are hypothesized as the result of genetic drift together with the population migration from Asia through Indonesian archipelago to Melanesia. Wahidiyat<sup>22</sup> reported that almost 50% of thalassemia syndrome in Jakarta and surroundings was compound heterozygote  $\beta^{Thal}/\beta^E$ . This figure was also reported by Purnomo Suryantoro<sup>23</sup> by molecular study in Yogyakarta.

### Clinical Variations

Clinically no problems arise from heterozygous  $\beta^E$  (trait); even in homozygous  $\beta^E/\beta^E$  no anemia or very mild anemia with microcytosis and hypochromia occur,<sup>3</sup> with Hb E as high as 80% in adults. But, interaction with  $\beta$ -thalassemia ( $\beta^{Thal}/\beta^E$ ) results in anemia with variable severity.<sup>5</sup> In severe cases the clinical manifestations and its problems are similar to homozygous  $\beta^{Thal}$ . The erythrocyte lifespan is shortened, around 7-15 days, and the erythrocytes are destroyed in the spleen in 60% of cases. Depending on whether it is  $\beta^{Thal}/\beta^E$  or  $\beta^{cThal}/\beta^E$ , Hb A is reported ranging from 46.8 $\pm$ 13.5 to 0 percent, Hb E 30.1 $\pm$ 12.2 to 42.7 $\pm$ 13 percent, and Hb F from 22.8 $\pm$ 7.2 to 57 $\pm$ 12.7 percent.<sup>4</sup> Like in thalassemia intermedia, many questions develop concerning the factor(s) affecting the severity of the of  $\beta^{Thal}/\beta^E$ . Not only the genotypes play role; in spite of seemingly identical genotypes, the severity of patients can greatly vary. A

spectrum of severity with Hb ranging from 2.5 to 13.9 g/dL has been observed in a population of 802 patients with  $\beta^{\text{Thal}}/\beta^{\text{E}}$ .<sup>5</sup>

Many genetic factors have been shown to determine the different severity of anemia in  $\beta^{\text{Thal}}/\beta^{\text{E}}$ . Mutations causing  $\beta^{\text{E}}$ -thalassemia usually result in some anemia whereas mutations causing  $\beta^{\text{E}}$  can produce either a severe or a mild clinical outcome.<sup>3</sup> A common sequence variation (T->C) at position -158 upstream of the  $\beta^{\text{E}}$ -globin gene detectable by restriction enzyme XmnI results in increased Hb F production, although Xmn I  $\beta^{\text{E}}$  negative might also have a high Hb F.<sup>24</sup> In  $\beta^{\text{E}}$ -Thal, this sequence was proved to be associated with milder anemia.<sup>25</sup> Two copies of these alleles are necessary to produce a significant clinical effect. Increased expression of  $\beta^{\text{E}}$ -globin gene and higher production of Hb F would help to reduce the overall globin chain imbalance and thus result in a less severe anemia. The -158(C->T) mutation is also associated with the mild thalassemia with -29(C->T) (TATA box) mutation in blacks.<sup>11,12</sup>

An underproduction of  $\beta^{\text{E}}$ -globin from  $\beta^{\text{E}}$ -globin gene strongly suggests that the consequence of alternative RNA splicing is of physiological significance. It affects the amount of the  $\beta^{\text{E}}$  in mRNA and hence determines the difference in severity of anemia in  $\beta^{\text{Thal}}/\beta^{\text{E}}$  patients who otherwise have the same genetic determinants. Winichagoon et al.<sup>26</sup> reported 8 patients  $\beta^{\text{4bpdel}}/\beta^{\text{Thal}}$  that showed Hb levels ranging from 4.9-10.6 g/dL, a great variation of Hb level among the same genetic mutations. Among them, three patients  $\beta^{17}/\beta^{\text{E}}$  were found with Hb 5.1-7.3 g/dL and three other patients  $\beta^{\text{IVS2,654}}/\beta^{\text{E}}$  with Hb 6.7-11.9 g/dL. The percentage of abnormal spliced  $\beta^{\text{E}}$ -globin mRNA in those patients were determined by RT-PCR (reverse transcription polymerase chain reaction) technique, and the result was that the severe patients had values of 2.9-6.1%, whereas in those with milder symptoms the value ranged between 1.6-2.6%. Based on the values and the Hb F level that varied and the Xmn I polymorphism (++, +- or --) the authors concluded that the extent of  $\beta^{\text{E}}$ -globin mRNA cryptic splicing was more associated with clinical severity of patients than did the patterns of Xmn I polymorphism at position -158 from  $\beta^{\text{E}}$ -globin gene or level of Hb F. However, the increased expression of  $\beta^{\text{E}}$ -globin gene and higher production of Hb F were also associated with the homozygosity of the Xmn I site (+/+) and this would help to result in a less severe anemia. From 802 patients studied by Fucharoen et al.<sup>5</sup> it was found that there was no significant difference between the level of Hb F in mild and in severe  $\beta^{\text{Thal}}/\beta^{\text{E}}$  cases (45.4 $\pm$ 15.08% vs 42.0 $\pm$ 11.54%) in spite of the fact that erythrocyte containing more Hb F survived longer.<sup>5</sup>

The manifestation of  $\beta^{\text{E}}$  is also reduced by the concurrence of  $\alpha$ -thalassemias.  $\beta^{\text{Thal}}/\beta^{\text{E}}$  coinheritant with  $\alpha$ -thalassemia was found to have Hb level of 7.4 g/dL or more, while those without  $\alpha$ -thalassemia had Hb levels either higher or lower than 7.4 g/dL.<sup>27</sup> Beta-thalassemia/HbE disease may show 10-13 g/dL Hb level if  $\alpha$ -thalassemia is concurrently present. In  $\beta$ -thalassemia the excess of  $\alpha$ -chains are precipitated in the cytoskeleton of red cells and some of these chains have become oxidized and the same process occurs on the protein 4.1 in  $\beta$ -thalassemia membrane.<sup>28</sup> The

concurrency  $\alpha$ -thalassemia with  $\beta$ -thalassemia reduces the production  $\alpha$ -globin and the decreased excess of  $\alpha$ -globin results in less precipitation of  $\alpha$ -globin in the erythrocyte membrane and thus less damage.<sup>5,10</sup>  $\alpha$ /non- $\alpha$  chain ratio in mild cases ( $2.08 \pm 0.42\%$ ) is significantly different from that in severe cases ( $2.55 \pm 0.34\%$ ). Therefore, the coinheritance of  $\alpha$ -thal-1 ( $-\alpha/-\alpha$ ) with  $\beta$ -thal may ameliorate the severity of manifestation more than  $\alpha$ -thal-2 ( $-\alpha/\alpha\alpha$ ), although many factors contribute their influence.<sup>29</sup> A 12% increase  $\beta$ -globin chain synthesis in  $\beta^{\text{othal}}$  mouse confers considerable protection against both oxidative damage and the consequent membrane instability in mouse  $\beta$ -thalassemia.<sup>30</sup>

The proteolytic cytosol activity in  $\beta^{\text{othal}}/\beta^E$  is proved to be higher than in the normal control, in the milder form it is higher than in the more severe form;<sup>31</sup> these suggest that the higher proteolytic activity in  $\beta$ -thalassemia destroys the excess of  $\alpha$ -globin chain more rapidly, leading to a less severe pathology in the red cells membrane, thus less anemia. Indeed, when human  $\alpha$ - and  $\beta$ -globin chain are used separately as substrates, it is found that the increased proteolytic activity in  $\beta$ -thalassemic erythrocyte is directed more toward the  $\alpha$ -globin chain. In the mild type of  $\beta^{\text{othal}}/\beta^E$  without concurrent  $\alpha$ -thalassemia (in which the excess  $\alpha$ -globin chain is higher) the proteolytic activity was higher than in the mild type with concurrent  $\alpha$ -thalassemia (in which the excess  $\alpha$ -globin chain is lower). The higher proteolytic activity must have been induced by the excess  $\alpha$ -globin chain, thus secondary to genetic factor.

Prenatal diagnosis has been implemented in the control of thalassemia with great success in some countries, reducing the birth of thalassemic babies to the level 10% of that without control.<sup>7</sup> The birth of the severe type thalassemia will be the more important target to be controlled if various types are exist. The study on genetic factor as the determinant of the variable severity of  $\beta$ -thalassemia/hemoglobin E disease is important when the prenatal diagnosis to be applied in countries where Hb E is of medical and community problem.

## Conclusion

Heterozygous Hb E gives no clinical problem, even homozygous gives no or mild anemia. But,  $\beta^{\text{othal}}/\beta^E$  gives severe to mild anemia. Many factors play role in determining the varied anemia resulted in, either genetic factors as well as biochemical processes. The study on genetic factor in  $\beta$ -thalassemia/hemoglobin E disease is important in the prenatal diagnosis.

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