CHAPTER IV

DISSCUSION

Thalassemia and hemoglobinopathies are the genetic disorders commonly found in tropical area including Thailand (1) in which α- and β-thalassemia are most frequently encountered. For Thailand, in particular, approximately 3-9% of its population carry the β-thalassemia gene and 13-70% carry that of the HbE. Molecularly, β-thalassemia rarely arises from the complete loss of a β-globin gene, but mutations could occur along the gene and affect the efficiency of β-globin production (21). On the clinical ground, it has been clear that the patients with β-thalassemia may suffer from severe disease as a results of ineffective erythropoiesis that leads to a life-threatening chronic anemia. However, these patients may also be suffering from varieties of complications that could fatally adverse the clinical course of the patients. Iron overload is one of the most important complication in patients with β-thalassemia and has long been a major focus of management (35). This condition may be caused by massive blood transfusion, chronic hemolysis and increased intestinal iron absorption due to expanded erythropoiesis and, possibly, the HFE mutations that are responsible for hereditary hemochromatosis (HH) (67).

Iron parameters used to evaluate the iron status include tests for serum iron (SI), total irom binding capacity (TIBC), transferin saturation (TS), serum ferritin, Hb levels and other red blood cell indicies, bone marrow biopsy and liver biopsy (68, 69). The simplest tests that indirectly give an indication of iron stores are the SI, TIBC and TS. The serum ferritin correlates well with iron store, but it can also be elevated with liver disease, inflammatory conditions and malignant neoplasms. However, it is still the gold standard. The red blood cell parameters will also give an indirect measure of iron stores, because the mean corpuscular volume (MCV) can be decreased with iron deficiency. The amount of storage iron for erythropoiesis can be quantified by performing an iron stain on a bone marrow biopsy. Excessive iron stores can be determined by bone marrow and by liver biopsy (68, 69).

ZPP is a metabolic intermediate of the hemoglobin synthetic pathway which accumulates in red blood cells when iron supply is limited. During periods of iron insufficiency or impaired iron utilization, zinc becomes an alternative metal substrate for ferrochelatase, leading to increased ZPP formation. Evidence suggests that this metal substitution is one of the first biochemical responses to iron depletion (iron deficient erythropoiesis), causing increased ZPP to appear in circulating erythrocytes (47).

In this study, ZPP, SI, TIBC, TS as well as other basic hematological parameters were determined to evaluate the iron status in the patients with β-thalassemia at Maharaj Nakorn Chiang Mai Hospital. In the investigation, the levels of these parameters were determined in the studied subjects before an evaluation of their differences in these 3 groups of subjects. For ZPP, it is found that ZPP in β-thalassemias was higher than those in the non-thalassemia indicating that the iron utilization in those β-thalassemic patients was impaired. This finding was concordant with those described by Tillyer *et al* in that ZPP levels raises in significant numbers of subjects with β and α-thalassemia(66). More importantly, mean ZPP levels in β-thalassemia/HbE was found to be higher than in those in the homozygous β-thalassemia patients also suggesting that iron utilization for erythropoiesis is less efficient in those β-thalassemia/HbE disease.

The study has also showed the evidence of iron overloading status in the β-thalassemia syndrome; the findings of which in agreeable with those previously evaluated (56, 66, 70). This was seen by the higher TS levels in the β-thalassemia/HbE disease and homozygous β-thalassemia than the non-thalassemic individuals.

The correlation between ZPP versus SI and TS levels was evaluated in non-thalassemia, β-thalassemia/HbE and homozygous β-thalassemia. Although correlation was not overwhelmed, the inverse relationship between ZPP versus SI and TS has demonstrated some meanings. The iron utilization in both types of β-thalassemia syndrome encountered at Maharaj Nakorn Chiang Mai Hospital might not be severely impaired in spite of iron overload. This might partly be a consequence of multiple blood transfusion in these patients that alleviates the degree of ineffective

erythropoiesis, a condition leading to the status so-called relative iron deficiency (54). Relative iron deficiency is a condition in which iron is delivered to the marrow at a rate insufficient to meet the demands of accelerated erythropoiesis (71). Examples where this may occur include ineffective erythropoiesis or hemolytic anemia cases in which iron requirements for erythrocyte production become exaggerated. Sideroblastic anemia is a metabolic defect in iron utilization that produces a deficiency-like response with increased ZPP (72). Impaired iron utilization is commonly found in anemia of chronic disease and leads to increased ZPP, which can be used to identify such anemias (73). As a rule, a greater proportion of hospitalized patients can be expected to have increased ZPP/H because of the numerous etiologies that impair iron utilization in the bone marrow. Despite this apparent lack of specificity, ZPP is very specific when defined in terms of marrow iron requirements rather than in terms of iron stores (serum ferritin) or the products of iron utilization (hemoglobin and hematocrit). Given a clear understanding and accurate interpretation of results, evidence shows that ZPP is a good screening tool for iron deficiency even in hospitalized patients (74). Thalassemia is characterized by a disordered globin chain formation. However, thalassemia also suggests iron deficiency by virtue of its characteristic low mean corpuscular volume. The latter is explained by an extreme erythroid hyperplasia in thalassemia that creates a state of relative iron deficiency (54). ZPP can be used to differentiate this apparent iron deficiency based on low mean corpuscular volume from that attributable to impaired hemoglobin (or globin) synthesis in thalassemia (47).

C282Y polymorphisms were not found in this studied cohort. This was not surprising as this polymorphism has been shown to be uncommon in Far East and Southeast Asia (75-80). H63D polymorphism, however, was not present in the 100 studied individuals. This was not as expected since this ancient polymorphism is supposed to be found worldwide (40, 58, 76, 77, 79-82). The finding in this study was concordant with that described by Viprakasit et al. Which found that only about 1% of the northern Thai population carry the H63D allele (83). The absence of H63D in this population might be in fact due to the small number of the subjects recruited in this study. The presence of HH, if any, might be a result of the other causes such as mutations in the gene encoding

hepcidin antimicrobial peptide (HAMP), mutations in the gene encoding transferrin receptor-2 and mutations in the SLC11A3 gene.

Blood samples were collected from non-thalassemic individuals, homozygous β-thalassemia and β-thalassemia/HbE disease. Hemoglobin identification was subsequently performed in weak cation exchange HPLC. Hb pattern in the non-thalassemic group was A_2A, that in homozygous β-thalassemia was A_2FA and that in the β-thalassemia/HbE disease was EFA. HbA that was found in all those being β-thalassemia/HbE disease and homozygous β-thalassemia although the molecular analysis revealed that all of them carried severe types of β-thalassemia mutations. The presence of HbA in these patients was most likely due to frequent blood tranfusions that these patients had been receiving.

Bilirubin is yellow pigment in the serum and can interfere with the measurement of ZPP under the priciple of hematofluorometry used in the portable ProtoFluor Z Hematofluorometer machine (84, 85). The association of total bilirubin and the ZPP levels (previously determined) were evaluated. ZPP in high total bilirubin group is higher than in total bilirubin group. For all samples, ZPP in washed samples is lower than in unwashed samples . This supports that bilirubin has affect to ZPP level. Thus before measuring ZPP ought to wash red blood cell by 0.85% NSS to deplete bilirubin.

In the study of Roh *et al.* (86), lead poisoning can cause an elevated ZPP levels. Thus in this study, determination of blood lead levels was performed in subjects who had high ZPP levels (> 80 umol ZPP/mol heme). All subjects had blood lead levels within normal range. Thus, raised ZPP levels in the patients should not be caused by lead intoxication, but was a true high ZPP quantity in the erythrocytes.