Zinc Protoporphyrin (ZPP) Status in Thai β-Thalassemia and Hb H Disease

Thanusak Tatu¹*, Weerasak Nawarawong² and Torpong Sa-nguansermsri³

¹ Division of Clinical Microscopy, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
² Division of Hematology, Department of Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
³ Thalassemia Research Unit, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

*Corresponding author. E-mail: asittt@chiangmai.ac.th

ABSTRACT

Zinc protoporphyrin (ZPP) is a compound formed by the coupling of zinc and protoporphyrin IX in the state when the body cannot utilize iron for heme formation. Thus, the raised levels of ZPP is the indicator of iron-deficient erythropoiesis. The objectives of this study were to determine the levels of ZPP in thalassemic patients with the long-term goal in the evaluation of iron-deficient erythropoiesis in these patients. In addition, the study was also aimed to assess the potential of ZPP as a marker for iron-overloading status, commonly observed in thalassemic patients. The study was conducted in 29 homozygous β-thalassemia, 34 hemoglobin E/β-thalassemia, 8 Hb H disease as well as 26 normal individuals. The ZPP was determined by the Hematofluorometric technique, iron parameters (SI, TIBC and TS) by the standard colorimetric method and red blood cell parameters by an automated blood cell analyzer. It was demonstrated that iron-overloading state occurred in thalassemic patients. The ZPP levels were significantly higher in thalassemic patients than in normal individuals. The highest levels of ZPP were seen in the HbE/β-thalassemia. Direct relationships were found between the ZPP levels and Hb, Hct, MCV, MCH and MCHC. However, inverse correlations were observed between ZPP levels and RDW and HbF levels. Finally, no correlations were revealed between ZPP levels and iron parameters, including SI, TIBC and TS. It was concluded that iron-deficient erythropoiesis is present in homozygous β-thalassemia, HbE/β-thalassemia and Hb H disease. The ZPP levels could not be used to predict the severity of iron-overloading status, but instead, was promisingly applicable in assessing the iron-deficient erythropoiesis in these three types of thalassemia.

Key words: Zinc protoporphyrin (ZPP), Iron-deficient erythropoiesis, Iron overload, Thalassemia, β-thalassemia, HbE/β-thalassemia, Hb H disease

INTRODUCTION

Hemoglobin, the major intraerythrocytic oxygen-carrying protein, is a compound consisting of heme and globin chains. Globin chains are the products of globin mRNA
translation. Heme is normally a compound generated from the coupling of Fe and protoporphyrin IX (Bunn et al., 1977; Weatherall and Clegg, 1981). However, in a condition where the iron utilization by erythroid tissue is impaired such as in iron-deficiency anemia or anemia of chronic disorders, zinc (Zn) will be incorporated into protoporphyrin IX and a new compound is resulted which is called zinc protoporphyrin or ZPP. Thus, the raised ZPP level is an indicator of absolute iron deficiency and iron-deficient erythropoiesis (Braun, 1999). In addition, ZPP levels are also increased in lead intoxication which, although under different mechanisms from previously-stated conditions, iron utilization is also impaired (Sassa et al., 1973; Lamola and Yamane, 1974; Labbe, 1977; Labbe et al., 1979; Trundle, 1984; Braun, 1999).

Thalassemia and hemoglobinopathies are the syndromes arising from quantitative and qualitative defects of globin chain synthesis, respectively (Weatherall and Clegg, 1981). Two types of thalassemia and hemoglobinopathies, α- and β-thalassemia, are commonly encountered worldwide. In Thailand, the frequency of α-thalassemia gene is about 20-30%, that of β-thalassemia gene is 3-9% and that of HbE is 13% with the highest frequency (50-60%) at the junction of Thailand, Loas and Cambodia (Wasi et al., 1969; Fucharoen & Winichagoon, 1997; Fucharoen et al., 1998). Those affected individuals generally have chronic anemia, requiring regular blood transfusion and always gradually become suffered from iron-overloading condition which generate several life-threatening complications (Weatherall and Clegg, 1981).

Iron-overloading status in thalassemic patients is generally evaluated by analyzing the levels of serum iron (SI), total iron binding capacity (TIBC), transferrin saturation (TS) and serum ferritin (Weatherall and Clegg, 1981). The levels of all these iron parameters except TIBC are much higher than normal in thalassemic individuals which is straightforward in those suffering from iron overload (Pootrakul et al., 1980; Pootrakul et al., 1981; Hsu et al., 1990; Siritorn et al., 1994; Hathirat et al., 1994). ZPP, although being one of iron parameters, has little been explored in thalassemic patients. However, one study performed by Hirsch and colleagues in 1991 demonstrated that the ZPP levels were elevated in sickle cell disease, indicating the impairment of iron utilization in these patients even though they were having iron overloading condition (Hirsch et al., 1991). In addition, Graham and co-workers in 1996 showed that the ZPP levels also increased significantly in α-, β- and HbE heterozygotes (Graham et al., 1996). These findings raised the question whether or not the iron utilization is also impaired in the more severe forms of thalassemia and hemoglobinopathies commonly found in Thailand.

The aims of the present study were to determine the ZPP status in Thai α- and β-thalassemia and to evaluate the correlation of the ZPP levels and iron parameters as well as the hematological parameters. The ultimate aim of this survey was therefore to demonstrate the status of iron utilization in these two types of thalassemia encountered in Thailand.

MATERIALS AND METHODS

The study was performed in 29 homozygous β-thalassemia, 34 hemoglobin E/β-thalassemia and 8 Hb H disease attending the Thalassemia Clinic, Department of Medicine, Faculty of Medicine, Chiang Mai University. Informed consent had been signed before recruitment. In addition, 26 normal individuals were also recruited to serve as normal control.
Blood samples were collected: 3 ml stored in EDTA for ZPP determination, hemoglobin identification and automated blood cell analyzer (Sysmex KX-21, Sysmex Corporation, Kobe, Japan) and 5 ml clotted blood for iron parameter determination. ZPP levels were evaluated by Hematofluorometric technique (Protoflor™) under manufacturer’s instruction (Helena Laboratories, Beaumont, Texas, USA). Hemoglobin identification was achieved by high-performance liquid chromatography (HPLC) technique (Primus Variant System99, CLC385™, Primus Corporation, Kansas City, MO, USA). Iron parameters including serum iron (SI), total iron binding capacity (TIBC) and transferin saturation (TS) were determined by standard colorimetric method recommended by the International Committee for Standardization in Hematology (ICSH) (Lewis, 1971). Those samples having ZPP levels more than 80 μmole/mole heme were subjected to lead level determination by the atomic absorption technique, using the Varian SpectrAA-400Z™ atomic absorption spectrometer. All the results were statistically analysed by the statistical software.

RESULTS

The levels of ZPP in thalassemic patients were significantly higher than in non-thalassemic individuals ($p < 0.05$). SI and TS levels were also significantly higher in β-hemoglobinopathies (homozygous β-thalassemia and Hb E/β-thalassemia) than in normal individuals ($p < 0.05$) whereas no differences between these parameters were observed between normal and Hb H disease. In contrast, the levels of TIBC were not different in the studied subjects (Table 1). However, when compared within the thalassemic patients, the values of all parameters were not significantly different except those of ZPP which were significantly higher in HbE/β-thalassemia than in homozygous β-thalassemia whereas HbE/β-thalassemia seemed to possess the highest ZPP levels as demonstrated in Figure 1-A. Although not significantly different, the highest levels of SI were seen in homozygous β-thalassemia (Figure 1-B), those of TIBC in Hb H disease (Figure 1-C) and those of TS in Hb E/β-thalassemia (Figure 1-D).

The relationships between ZPP and hematological parameters including RBC count, Hb, Hct, MCV, MCH, MCHC, RDW and Hb F level were evaluated with 95% confidence interval. Modest but significantly-negative correlation was found between ZPP and all hematological parameters except the RDW and Hbf levels of which the relationships with ZPP were in positive trend (Figures 2A-2H).

The relationships between ZPP levels and iron parameters including serum iron (SI), total iron binding capacity (TIBC) and transferrin saturation (TS) were evaluated at the confidence interval of 95%. No relationships were observed between all these iron parameters and ZPP as shown in Figures 3A-3C.
Table 1. ZPP, SI, TIBC and TS levels in homozygous β-thalassemia (β/β), Hb E/β-thalassemia (β/E), Hb H disease (Hb H Dis) and normal individuals (N). The values are expressed as mean ± SD and “n” represents numbers of subjects.

<table>
<thead>
<tr>
<th></th>
<th>ZPP (umole/mole Heme)</th>
<th>SI (ug/dl)</th>
<th>TIBC (ug/dl)</th>
<th>TS (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>35.1±15.5</td>
<td>124±24</td>
<td>280±60</td>
<td>46±14</td>
<td>26</td>
</tr>
<tr>
<td>β/E</td>
<td>80.5±31.4</td>
<td>182±87</td>
<td>242±113</td>
<td>78±25</td>
<td>34</td>
</tr>
<tr>
<td>β/β</td>
<td>61.9±21.3</td>
<td>224±117</td>
<td>301±167</td>
<td>77±26</td>
<td>29</td>
</tr>
<tr>
<td>Hb H Dis</td>
<td>77.0±33.5</td>
<td>191±109</td>
<td>319±198</td>
<td>62±23</td>
<td>8</td>
</tr>
<tr>
<td>N</td>
<td>30-80</td>
<td>M :50-160</td>
<td>F : 45-150</td>
<td>250-400</td>
<td>20-50</td>
</tr>
</tbody>
</table>

Figure 1. Comparisons of ZPP (A), SI (B), TIBC (C), TS (D) between normal (1), HbE/β-thalassemia (2), homozygous β-thalassemia (3) and HbH disease (4). [p-values (ZPP) : 1 vs 2 = 0.00, 1 vs 3 = 0.00, 1 vs 4 = 0.01, 2 vs 3 = 0.01, 2 vs 4 = 0.78 and 3 vs 4 = 0.13] [p-values (SI) : 1 vs 2 = 0.00, 1 vs 3 = 0.00, 1 vs 4 = 0.13, 2 vs 3 = 0.11, 2 vs 4 = 0.81 and 3 vs 4 = 0.48] [p-values (TIBC) : 1 vs 2 = 0.12, 1 vs 3 = 0.53, 1 vs 4 = 0.60, 2 vs 3 = 0.11, 2 vs 4 = 0.14 and 3 vs 4 = 0.80] [p-values (TS) : 1 vs 2 = 0.00, 1 vs 3 = 0.00, 1 vs 4 = 0.10, 2 vs 3 = 0.92, 2 vs 4 = 0.13 and 3 vs 4 = 0.16].
Figure 2. Correlations of ZPP levels and RBC count (A), Hb (B), Hct (C), MCV (D), MCH (E), MCHC (F), RDW (G), HbF (H).
Figure 3. Correlations of ZPP levels and SI (A), TIBC (B), TS (C).

DISCUSSION AND CONCLUSION

Zinc protoporphyrin (ZPP) is a substance originated from the incorporation of Zn\(^{++}\) instead of Fe\(^{++}\) into protoporphyrin IX during the stage of erythropoiesis (Braun, 1999). Elevated levels of ZPP reflect the status of impairment of iron utilization owing to both conditions (Lamola and Yamane, 1974; Joselow and Flores, 1977a; Joselow and Flores, 1977b; Labbe, 1992; Hinchliffe et al., 1995; Kim et al., 2003). Another form of protoporphyrin, the Free Erythrocyte Protoporphyrin (FEP), was initially measured to evaluate the state of impaired iron utilization and direct relationship was observed between the values of FEP and ZPP (Joselow and Flores, 1977b; Karacic et al., 1980; Vichinsky et al., 1981; Tatu et al., 1996). In thalassemia field, iron-overload has for a long time been recognized as a life-threatening complication. In fact, in iron overloading status, the levels of ZPP or FEP should not rise because iron supply for erythropoiesis should already be enough. However, several reports have obviously demonstrated that FEP levels increased significantly in sickle cell disease, β-thalassemia and HbH disease, indicating that impairment of iron utilization has occurred in these conditions (Vichinsky et al., 1981; Pootrakul et al., 1984). So far, the informations about ZPP status in the thalassemia disease have not been substantially reported. In contrast, ZPP levels were evaluated in α-thalassemia heterozygote, β-thalassemia heterozygote and HbE heterozygote (Han et al., 1990; Tillyer and Tillyer, 1994; Graham et al., 1996). Elevated ZPP levels were revealed in all types of thalassemia heterozygotes they analyzed which could also implicate the impairment of iron utilization.

In the present study, we evaluated the ZPP levels in homozygous β-thalassemia, HbE/β-thalassemia and HbH disease, all of which cases reside in the northern part of Thailand. Iron status measured as SI, TIBC and TS was also evaluated as well as the hematological parameters to determine their relationships with the ZPP levels. As expected, iron overload
was evidently demonstrated in all thalassemic individuals. The ZPP levels were also increased dramatically in thalassemic patients. This finding emphasized an existence of the ineffective iron utilization or relative iron deficient state in thalassemic patients among the atmosphere of iron overload, the identical picture that has already been shown previously by Pootrakul et al., (1984). This impairment of iron utilization in these thalassemia might be a result of ineffective and expanded erythropoiesis in compensation to the bodies’ requirement of erythrocytes, a condition formerly termed “relative iron deficiency” by Pootrakul et al (1984). This phenomenon was emphasized by the inverse relation between ZPP levels and RBC, Hb, Hct, MCV, MCH and MCHC levels and direct relation between ZPP levels and RDW and HbF levels. Where ineffective erythropoiesis occurs, RBC mass reduces as well as other red blood cell parameters. On the other hand, accelerated erythropoiesis results in the release of red blood cell with different size and seen as a broad range of RDW (Weatherall, 1998). HbF has been well proven to be increased in β-thalassemia (Wood, 1993). Elevated HbF levels in β-thalassemia may be a result of selective survival of young erythrocytes, prematurely released in compensation to anemia that are still capable of γ-globin chain production (Wood et al., 1980; Wood, 1993; Weatherall, 1998). The correlation of ZPP levels with all these hematological parameters was meaningful in indicating the presence and degree of severity of ineffective erythropoiesis. Iron overload, although significantly evident in the studied subjects, did not correlate well with the ZPP levels. Indeed, iron overload is a consequence of multiple blood transfusions and increased intestinal absorption and accumulate in varieties of vital organs (Weatherall, 1998). The information obtained from the present study showed that it did not seem to have direct interaction with the formation of ZPP in these patients. Thus the ZPP levels cannot serve as a good predictor or marker for the degree of iron overload. In conclusion, iron-deficient erythropoiesis was present in homozygous β-thalassemia, HbE/β-thalassemia and HbH disease. The ZPP levels could not be used to predict the severity of iron-overloading status, but instead, was promisingly applicable in assessing the iron-deficient erythropoiesis in these three types of thalassemia.

As raised levels of ZPP might be due to lead intoxication (Lamola and Yamane, 1974), the samples with ZPP level higher than 80 μmole/mole heme were subjected to lead level determination by atomic absorption spectrometry. Blood lead levels were within normal range in all subjects tested. The ZPP level is also affected by the presence of bilirubin in blood samples (Buhrmann et al., 1978). To avoid this problem, all blood samples were washed with normal saline solution prior to determination of ZPP by the Hematofluorometer.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of Hematology Clinic and Hematology Laboratory, Faculty of Medicine, Chiang Mai University and Maharaj Nakorn Chiang Mai Hospital in collecting blood samples. The deep gratitude is also put forward to the Research Center for Hematology and Health Technology, Division of Clinical Microscopy, Department of Medical Technology, Chiang Mai University in supporting the space, equipment for this study. This study was financially supported by the National Budget for the fiscal year 2545 BE.
REFERENCES
