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# FREE ERYTHROCYTE PORPHYRINS (FEP) IN NORMAL ADULTS AND THALASSEMIC CHILDREN

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## ABSTRACT

The level of free erythrocyte porphyrins (FEP) was determined by the micromethod of Piomelli in 46 normal adult males, 12 pregnant women and 31 children with homozygous beta-thalassemia and beta-thalassemia Hb.E. disease. The FEP level of 46.34 ± 16.00 (range 15.0-76.5) ug/100 ml red cells in normal adult males was similar to those recently observed by many investigators but slightly higher than those of older reports. This can be explained by the fact that with the present micromethod used the extraction of FEP is more efficient and complete than the older methods. The FEP level of 60 36 ± 16.28 (range 37.2-87.2) ug/100 ml red cells in pregnant women was significantly higher than normal adult male value ($p < 0.01$) and can be explained on the basis of sex difference and the low transferrin saturation. The FEP level of 91.31 ± 51.13 (range 31.3-225.0) ug/100 ml red cells in 31 thalassemic children further indicated that in addition to the primary defect in globin chain synthesis the heme synthesis is also impaired.

Porphyrins are widely distributed in living cells and play essential roles in various metabolic processes such as photosynthesis, transportation of oxygen and cellular respiration. The most important porphyrin in human is

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protoporphyrin 9, type III, which in combination with iron and specific proteins forms such compounds as hemoglobin, cytochrome, peroxidase and catalase. In red cells, in addition to those incorporated into heme small amount of protoporphyrin (less than 1 mole to 24,000 moles of heme) is found in apparently uncombined or free form (free erythrocyte protoporphyrin or FEP) along with smaller quantities of other porphyrins, primarily coproporphyrin [1, 2]. Since protoporphyrin is the last intermediate in the biosynthesis of heme increased concentration of FEP is found in various disorders in which the protoporphyrin is not efficiently utilized for heme synthesis, as in erythropoietic porphyrias [3], sideroblastic anemia [4], iron deficiency [1, 5-7], lead intoxication [6, 8-11], and anemia of chronic disorders such as chronic infection [12. 13] and rheumatoid arthritis [14, 15]. It seems likely, with the exception of primary disorder in porphyrin synthesis, that the common denominator of increased FEP was an iron supply or incorporation inadequate to meet the needs of the erythroid marrow.

The fundamental defect of thalassemia syndrome is the defective synthesis of globin chains. Nevertheless, accumulated evidences including an increased FEP [16] and the activity of heme enzyme delta-ALA dehydratase [17] in red cells, and the abnormally increased urinary excretion of PBG, coproporphyrin and dipyrroles [18, 19] strongly indicated that pyrrole metabolism and heme synthesis of thalassemic patients are also disturbed [20-23]. The conflicting results regarding the FEP levels in patients with thalassemia major and minor obtained by various investigators [16.20,24-26] can be partly explained by the differences in methodology and in the FEP values of control population. The conventional spectrophotometric methods [27,28] of determination of FEP involve exhaustive extraction with organic solvents and re-extraction from the solvent phase with hydrochloric acid which is not only time consuming but also requires large amount of blood sample. The micromethod recently described by Piomelli [11] is simple, rapid and the extraction of FEP is more efficient and complete than in other methods. With the aid of this technique, the status of FEP levels in our thalassemic children were evaluated.

## MATERIALS AND METHODS

Heparinized venous blood samples were obtained from 46 healthy adult male, 12 pregnant women and 31 children with homozygous beta-thalassemia and beta-thalassemia Hb E disease. After a portion of plasma was separa-

ted the volume of packed red cells of each blood sample was determined by the standard microhematocrit technique.

The values of free erythrocyte porphyrins (FEP) were determined by the microfluorometric procedure of Piomelli[11] with slight modification. One tenth ml of the anticoagulated whole blood sample was transferred into a tube containing 5 ml of 5% celite suspension (in 0.9% NaCl) and mixed. After 5 ml of a 4:1 mixture of ethylacetate/acetic acid was added the tube was agitated vigorously on a vortex mixer for 60 seconds and centrifuged at 1,500 RPM for 3 minutes. The supernatant fluid was separated. Five ml of 1.5 N. HCl was added to the supernatant fluid and the tube was agitated on a vortex mixer for 60 seconds. An aliquot of the lower (colorless) HCl phase was transferred into a cuvette and the concentration of FEP was determined by reading the % emission in the fluorometer calibrated with a series of standard coproporphyrin I solutions. A blank is prepared in parallel by replacing the whole blood sample with 0.1 ml of saline solution. The final concentration of FEP was calculated from the formula:

$$\text{FEP ug/100 ml RBCs} = \frac{\text{FEP (ug/100 ml blood)}}{\text{Hct.}} \times 100$$

The standard solution of porphyrin (coproporphyrin I) was prepared by diluting the standard coproporphyrin I (5 ug/vial) with 2.5 ml of 0.1 N. HCl and heated for 5 minutes in boiling water. The serial dilutions were made from this stock solution (original concentration was 200 ug/100 ml). The example of standard calibration curve is shown in Figure 1.

## RESULTS.

The results of our study are summarized in Table 1. The FEP value of 46.34 ± 16.0 ug/100 ml red cells in our normal adult males is similar to those recently found by the others[10, 11, 26, 29]. The FEP value of 60.36 ± 16.28 ug/100 ml red cells in pregnant women is significantly higher than normal adult male ($p < 0.01$). The FEP values of both male and female thalassemic children are definitely higher than normal adult value ($p < 0.001$). The difference between FEP values of these two groups of children is not statistically significant ($p > 0.05$). Figure III illustrated the individual value of FEP level in thalassemic children. The dotted lines represent the normal adult level (95% reliability).

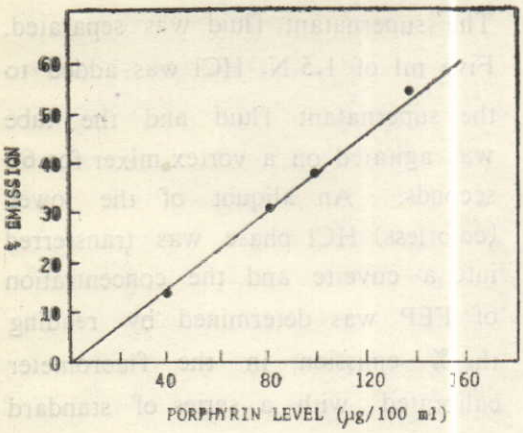


FIGURE I. STANDARD PORPHYRIN CALIBRATION CURVE.

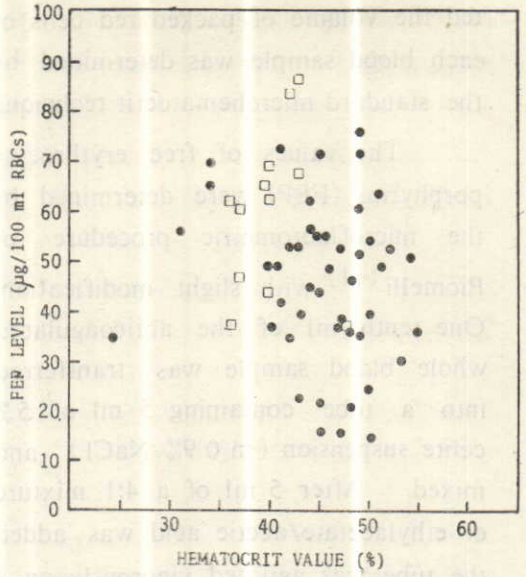


FIGURE II. FEP LEVELS IN NORMAL ADULT MALES (●) AND PREGNANT WOMEN (□).

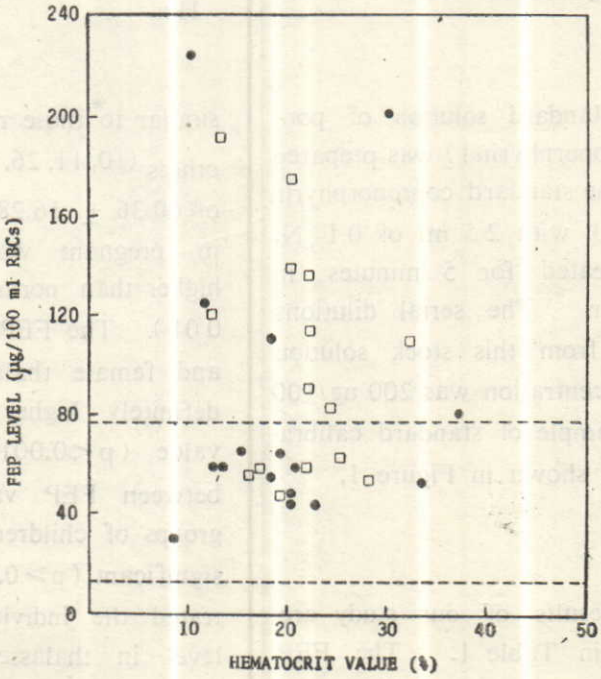


FIGURE III: FEP LEVELS IN MALE (●) AND FEMALE (□) BETA THALASSEMIA CHILDREN

**Table 1.** Values of Free Erythrocyte Porphyrins (FEP) in Thai Subjects.

| SUBJECTS | No. | AGE yr. | HCT % | FEP ug/100 ml. RBCs |
|---|---|---|---|---|
| Normal Adult Males | 46 | 23.4 ± 6.8 | 45.2 ± 5.9 | 46.34 ± 16.00 (15.0-76.5) |
| Pregnant Women | 12 | 27.3 ± 12.6 | 40.0 ± 3.3 | 60.36 ± 16.28 (37.2-87.2) |
| Thalassemic Children | 31 | 7.6 ± 3.6 | 20.1 ± 6.8 | 91.31 ± 51.13 (31.3-225.0) |
| : Male | 16 | 8.1 ± 4.1 | 19.3 ± 8.2 | 83.34 ± 55.93 (31.3-225.0) |
| : Female | 15 | 7.1 ± 3.8 | 20.9 ± 5.2 | 99.81 ± 45.83 (47.4-192.0) |

**Table 2.** Values of Free Erythrocyte Porphyrins (FEP) in Thalassemic Subjects.

| AUTHORS | No. | FEP ug/100 ml. RBCs | REFERENCE |
|---|---|---|---|
| THALASSMIA MINOR | | | |
| : Sturgeon et al (1955) | 9 | 22 - 75 | (24) |
| : Sturgeon et al (1958) | 7 | 31 | (25) |
| : Ludin (1962) | 10 | 60 - 438 | (20) |
| : Lyberatos et al (1972) | 31 | 70.4 ± 22.19 (44-108) | (16) |
| : Stockman et al (1975) | 29 | 38 ± 14 (11-72) | (26) |
| THALASSEMIA MAJOR | | | |
| : Schwartz-Tiene (1953) | 10 | 96 - 168 | (20) |
| : Sturgeon et al (1955) | 7 | 19 - 110 | (24) |
| : Sturegon et al (1958) | 10 | 36 | (25) |
| : Lyberatos et al (1972) | 20 | 83.3 ± 23.42 (55-156) | (16) |
| : Present Study (1978) | 31 | 91.31 ± 51.13 (31.3-225.0) | |

## DISCUSSION

Our mean FEP values in normal adult males is similar to those observed by many investigators [10,11,26,29] but somewhat higher than those found by the others [1]. Since there is no breakdown of heme to protoporphyrin occurred in this technique the possible explanation lies in the fact that the extraction of FEP by the very large solvent-erythrocyte ratio is more efficient and complete than in older methods. The higher FEP levels in pregnant women can be explained on the basis of sex difference [30] and most probably due to relative iron deficiency [1]. A portion of the normal protoporphyrin does appear to be related to iron deficiency state. In normal subjects, a significant correlation between transferrin saturation and protoporphyrin level is observed. The protoporphyrin level is somewhat higher with lower transferrin saturation. In pregnant women, the progressively decreased transferrin saturation results in accumulation of protoporphyrin in red cells because the nonavailability of iron prevents conversion of protoporphyrin 9 to heme [1].

The elevation of FEP level in beta - thalassemic red cells may be explained by the fact that although its primary defect involves the globin chain synthesis, there is impairment of heme synthesis as well. The synthesis of heme and globin are closely linked [31]. Defective globin synthesis results in a decreased heme synthesis through a feed back control mechanism [32] while the porphyrin synthetic enzyme delta-ALA dehydratase is increased [17]. The experimental study of porphyrin production indicated that when red cells or hemolysates are incubated in vitro free porphyrins tend to accumulated. Hemolysates of thalassemic red cells showed rapid incorporation of ALA into protoporphyrin indicating that the enzymatic steps between the two are intact [33]. Thalassemic hemolysates tended to accumulated a greater proportion of protoporphyrin than non - thalassemic hemolysates, suggesting a block in hemoglobin synthesis beyond the formation of protoporphyrin [21, 33, 34]. The question whether this is actually reflects incompleteness of hemoglobin synthesis or because of the preponderance of young cells in thalassemia has been raised [20]. It is now known that protoporphyrin exists within the red cell in a stable form, is not increased with a young population of cells and that reticulocytes per se do not have an increased protoporphyrin concentration [1]. Reports of the association between elevated protoporphyrin level and reticulocytosis [2, 35-39] can be explained by relative iron deficiency [1].

The acid-extracted porphyrins from red cell of normal individuals as well as those from the patients with primary porphyrias (erythropoietic protoporphyria or EPP) and secondary erythrocyte porphyrias (including lead intoxication, iron deficiency anemia etc.) are all chemically, physically, and spectrally identical to pure protoporphyrin-9 in acid aqueous solution (40). It has been incorrectly assumed that the natural state of the FEP in EPP and in other secondary porphyrias was identical. The detailed spectrofluorometric studies disclosed two distinct protoporphyrin species in this group of patients (40). The first species which is found in large quantities in red cells of patients with lead intoxication, iron deficiency anemia and chronic infection (and present in a very small amount in red cells of normal persons, patients with porphyria cutanea tarda and EPP) is the globin-bound zinc - protoporphyrin 9 (41). Acid-extraction of this protoporphyrin results in the loss of the chelated zinc from the protoporphyrin moiety. The resultant product exhibits spectral identity with the second species of protoporphyrin, metal-free protoporphyric as found in EPP patients. There are several other differences in the nature of these protoporphyrin species which are obscured by chemical changes occurring during extraction process. These differences may be part of the explanation for the difference in cutaneous photosensitivity between EPP, who are exquisitely light sensitive, and iron deficiency, lead intoxication and thalassemia patients who are not at all light sensitive.


## บทคัดย่อ

ผลการศึกษาปริมาณของ Free erythrocyte porphyrins (FEP) ในชายไทย หญิงตั้งครรภ์และเด็กที่ป่วยเป็นโรค thalassemia syndrome ด้วยวิธี microfluorometric technique ของ Piomelli ปรากฏว่าชายไทยมีสุขภาพสมบูรณ์ 46 คน มีระดับของ FEP ใกล้เคียงกับที่เคยมีผู้รายงานไว้หลายราย คือ 46 34 ± 16.0 (15.0–76.5) ug/dl red cells ค่าที่ได้นี้สูงกว่าการวัดด้วยวิธีเก่าเล็กน้อย เนื่องจากวิธีใหม่ที่ใช้นี้สามารถแยก FEP ออกมาจากเม็ดเลือดแดงได้ดีกว่าวิธีอื่น หญิงตั้งครรภ์ 12 คน มีระดับ FEP 60.30 ± 16.28 (37.2 - 87.2) ug/dl red cells ซึ่งสูงกว่าชายที่มีสุขภาพสมบูรณ์อย่างมีนัยสำคัญ ($p < 0.01$) ผู้ป่วยเด็กที่เป็นโรค beta-thalassemia ทั้งชนิด homozygous และชนิดเกิดร่วมกับ Hb. E disease จำนวน 31 คน มีระดับ FEP สูงมาก ถึง 91.31 ± 51.13 (31.3-225.0) ug/dl. red cells ($p < 0.0001$) ซึ่งสนับสนุนว่าผู้ป่วยที่เป็น beta–thalassemia นั้นนอกจากจะมีความผิดปกติในการสร้าง globin chain แล้วยังมีความผิดปกติในการสร้าง heme ด้วย.

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