# Erythrocyte Zinc Protoporphyrin in Beta-Thalassaemia Carriers

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## ABSTRACT

**Introduction**: In Malaysia, 4.5% of the population are carriers of beta-thalassaemia and a moderately high prevalence of iron deficiency has been reported. Both these conditions have red cells that are hypochromic and microcytic. Serum ferritin has been used to determine the status of iron storage and reduced levels are seen in iron deficiency. Serum ferritin, however, is an acute phase reactant and levels may increase in tissue necrosis and inflammatory disease. In iron deficiency, the enzyme ferrochelatase catalyses the incorporation of zinc, instead of iron into porphyrin IX, resulting in the formation of zinc protoporphyrin (ZnPP). **Objective**: To determine the erythrocyte zinc protoporphyrin levels in beta-thalassaemia carriers as a surrogate marker of functional iron deficiency. **Methods**: Automated blood counts and zinc protoporphyrin (ZnPP) were determined in 57 beta-thalassaemia carriers. The assay of ZnPP was done on washed red blood cells using a haematofluorometre. **Results and Discussion**: Nineteen (33.3%) of beta-thalassaemia carriers had raised ZnPP levels indicating the presence of functional iron deficiency. **Conclusion:** Both iron deficiency and beta-thalassaemia carriers have hypochromic red cells and iron deficiency that can co-exist in a carrier with beta-thalassemia. Individuals with this finding may benefit from iron supplementation.

**Keywords: Erythrocyte zinc protoporphyrin, beta-thalassaemia carrier, iron deficiency**

## INTRODUCTION

Iron deficiency anaemia is highly prevalent in many developing countries affecting 10 to 50% of the population in some areas.[1] In order to alleviate this nutritional deficiency, additional iron must be supplied to the population either with the diet (iron fortification) or as iron medication (iron supplementation). However, a significant concern is that some of these individuals may be adversely affected by the higher iron intake. For example in Malaysia where iron deficiency is common, 4.5% of the population have an inherited abnormality in haemoglobin synthesis. [2] Both these conditions are associated with a hypochromic microcytic anaemia making it difficult to distinguish one from another with blood counts generated from automated blood counters.[3]

Functional iron deficiency is a defined as failure to supply iron at a sufficient rate for haemoglobin synthesis in maturing red blood cells. The iron storage may be low (true iron deficiency) or normal/high (relative iron deficiency). Serum ferritin is used as a marker of

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iron status as it relates well to iron stores. Its limitation is that an increase in serum ferritin is caused by the acute phase responses associated with chronic inflammatory processes and in tissue necrosis.[4,5]

Protoporphyrin normally occurs in erythrocytes in very low concentrations. Most of this protoporphyrin IX is combined with iron to form heme. The rest of the porphyrin exists as zinc protoporphyrin (95%) and as free erythrocyte porporphyrin (5%). In iron depletion, the pathway for heme synthesis is interrupted and zinc is substituted for iron in protoporphyrin IX. Zinc protoporphyrin (ZnPP) accumulates in developing erythrocytes during functional iron deficiency.[6] In view of the current concerns of the optimal use of supplemental iron and the limitation of serum ferritin as a marker of iron status, ZnPP was measured in carriers of thalassaemia as a surrogate marker a of functional iron deficiency.

## METHODS

The study group consisted of 57 subjects who were carriers of classical beta-thalasaemia ($\beta^0$).[7,8,9] Classical beta-thalassaemia carriers are identified by normal or mildly reduced haemoglobin levels, erythrocytosis, microcytic and hypochromic red cell indices. All subjects had been analysed by conventional haematological and DNA characterisation as beta-thalassaemia carriers.[10,11,12] Blood samples (3.5 ml) were taken by venepuncture from the cubital fossa and introduced into ethylenediaminetetraacetate (EDTA) for haematological studies and for erythrocyte zinc protoporphyrin assays. The control group consisted of 45 healthy blood donors with no anaemia, normal red cell indices and serum ferritin levels >12 $\mu$g/L.

Red blood cell counts and red cell indices were collected in an automated blood cell counter (Coulter STKS, Coulter Corporation, USA). The parameters generated were haemoglobin (Hb), red cell count (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW). Zinc protoporphrin (ZnPP) was assayed using a front-faced haematofluorometre (Proto Fluor System, Helena Laboratories, Beaumont, Texas, USA). The red cells were washed twice with a 9 g/L sodium chloride solution prior to the assay to remove plasma interference. One drop of blood was mixed with 3 drops of Protofluor™ reagent. Measurements were made within 6 hours of taking the blood sample and the results expressed as a molar ratio of ZnPP to heme ($\mu$mol ZnPP/mol heme).

## RESULTS

1 Normal control group: 45 subjects were studied. Their ZnPP levels were <50 $\mu$mol/mol heme.

2 Beta-thalassaemia carriers (Table 1): 57 subjects were studied. Nineteen (33.3% had raised ZnPP levels (>50 $\mu$mol/mol heme). A positive correlation between ZnPP and RDW ($r=0.5$, $p<0.05$) was noted. Seven (12.3%) of the beta-thalassaemia carriers had haemoglobin (Hb) levels <10 gm/dl whereas only 4(57.1 %) had raised ZnPP levels.

This indicates that not all subjects with Hb <10 gm/dl have iron deficiency.

**Table 1**. Haematological data and erythrocyte zinc protoporphyrin levels in beta-thalassaemia carriers (n=57)

| Parameter | Mean±SD | Normal |
|---|---|---|
| ZnPP µmol/mol heme | 47 ± 11.1 | 23 - 49.9 |
| Hb g/dl | 11.4 ± 2.1 | 12 - 18 |
| RBCX$10^{12}$/L | 5.8 ± 0.7 | 3.8 - 6.5 |
| MCV fL | 64.8 ± 3.9 | 80 - 100 |
| MCH pg | 19.9 ± 1.4 | 27 - 32 |
| MCHC g/dl | 30.8 ± 1.7 | 32 - 34 |
| RDW % | 15.8 ± 1.6 | <14.8 |

ZnPP: zinc protoporphyrin; Hb: haemoglobin; RBC: red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width.

## DISCUSSION

The adequacy of iron supply for erythropoiesis was examined with zinc protoporphyrin (ZnPP) determinations in red blood cells from beta-thalassaemia carriers. The elevation of ZnPP levels is indicative of defective heme biosynthesis: it is a surrogate marker of functional iron deficiency. Raised ZnPP indicates that the iron being delivered in the bone marrow to developing red blood cells is defective. ZnPP determinations in combination with red blood cell indices have been employed in the screening programmes for various disorders. Latent iron deficiency and iron deficiency anaemia both result in abnormal ZnPP levels. Data routinely produced by automated blood counter analysers have been widely used as an aid to differentiate two common causes of microcytosis (MCV <80 fl) and hypochromia (MCH<27 pg) iron deficiency anaemia and thalassaemia.[13,14]

Discriminant analysis identified mean corpuscular haemoglobin (MCH), red cell count (RBC), mean corpuscular volume (MCV) and red cell distribution width (RDW) as the best set of indices for differentiating hypochromic conditions. The RDW is normal in uncomplicated thalassaemia carriers but raised in iron deficiency. In thalassaemia carriers with concurrent iron deficiency, the RDW will be raised.

Assessment of iron status is important, because iron deficiency and overload have pathological consequences. Serum ferritin is frequently used to assess iron stores for the purpose of ensuring their adequacy for erythropoiesis.[4] Both iron deficiency and thalassaemia have hypochromia (MCH <27 pg) and microcytosis (MCV<80 fl) in red blood cells. Beta-thalassaemia carriers can co-exist with true iron deficiency. Since iron absorption in carriers with thalassaemia is similar to normal subjects, a similar prevalence of iron deficiency would be expected. Thalassaemia carriers are likely to have true iron deficiency (serum ferritin levels <12 $\mu$g/L) rather than relative functional iron deficiency (serum ferritin levels >12 $\mu$g/L). The limitation of the serum ferritin assay is that measurable ferritin levels can be increased when tissue ferritin is released during cellular injury and with erythropoietic-reticuloendothelial blockade in chronic disease processes where failure to release iron to

developing red cells occurs. This results in serum ferritin estimations not reflecting the true iron status in the body.[4,5] When used in conjunction with MCH, ZnPP determinations can separate thalassaemia carriers and iron deficiency. Both iron deficiency and thalassaemia have hypochromia (MCH <27 pg) and microcytosis (MCV<80 fl) but in uncomplicated carriers of thalassaemia, the ZnPP levels are normal

## CONCLUSION

An increased level of erythropoietic zinc protoporphyrin (ZnPP) is a useful surrogate marker to identify iron deficiency in beta-thalassaemia carriers. Beta-thalassemia carriers can have concurrent iron deficiency and their identification leads to optimal use of supplemental iron therapy.

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