Effect of Iron Deficiency on the Phenotype of $\beta$-Thalassaemia Trait

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ABSTRACT

The objective of this study was to determine the effect of iron deficiency on Hb-A$_2$ level in $\beta$-thalassaemia trait and to determine the frequency of individuals with $\beta$-thalassaemia trait who could be missed due to concomitant iron deficiency. A total of 120 patients were studied, out of which 23 were iron deficient (serum ferritin < 20 ng/ml). Mean Hb-A$_2$ in the iron deficient individuals was 4.1 ±0.47% as compared to 5.1 ±0.58% in the remaining 97 individuals without iron deficiency (p < 0.001). In the 120 individuals with $\beta$-thalassaemia trait, mean Hb-A$_2$ was 5.8% with range 3 - 6.8% and confidence interval was 95%. In 2 individuals with $\beta$-thalassaemia trait, Iron deficiency was observed and showed Hb-A$_2$ less than 3.5%. These could have been missed while screening by Hb-A$_2$ estimation alone. Co-existence of Iron deficiency and $\beta$-thalassaemia trait may mask the diagnosis of beta thalassaemia trait and such individuals can be missed during screening by Hb-A$_2$ estimation alone.

Key Words: $\beta$-thalassaemia trait. Iron deficiency. Hb-A$_2$.

Thalassaemia is among the most common hereditary disorders worldwide. Thalassaemia is prevalent in the Mediterranean region, Africa, Middle East, Indian Subcontinent and South-East Asia. It is estimated that each year approximately 5000 new births of thalassaemia major take place in Pakistan. Marriage between two thalassaemia carriers can result in the birth of children with homozygous form of disease that requires lifelong blood transfusion. The birth of thalassaemia major patients can be prevented by carrier screening and prenatal diagnosis. Internationally acclaimed local study has highlighted that in Pakistan the gene variants are trapped within extended family groupings.

Beta thalassaemia carriers may have mild anemia with hypochromia, microcytosis, target cells and basophilic stippling. Their diagnosis is confirmed by Hb-A$_2$ estimation (level > 3.5%) after electrophoresis or HPLC. The expected rise of Hb-A$_2$ in $\beta$-thalassaemia trait can be masked by co-existing iron deficiency. This could result from decrease in transcription and/or translation of the globin genes or competition between $\beta$-globin chains (Hb-A) and $\sigma$-globin chains (Hb-A$_2$) in binding the limited quantity of available iron. The diagnosis of $\beta$-thalassaemia trait can be missed in case of concomitant iron deficiency if $\beta$-thalassaemia trait is screened by measuring Hb-A$_2$. This study was conducted to determine the effect of iron deficiency on the level of HbA$_2$ in $\beta$-thalassaemia trait and find the proportion of individuals who could be missed while screening for thalassaemia in the Pakistani setting where iron deficiency and thalassaemia are common.

This cross-sectional study was conducted from July 2011 to January 2012 at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi. The subjects of the study were picked by non-probability consecutive sampling method from the couples referred for prenatal diagnosis of thalassaemia. Their diagnosis was confirmed by PCR for $\beta$-thalassaemia mutations. Blood counts were performed on Sysmex KX-21 automated haematology analyzer. Haemoglobin electrophoresis of the supernatant lysate was performed on cellulose acetate membrane soaked in Tris EDTA borate buffer at pH 8.9. Negative and positive controls for $\beta$-thalassaemia trait were also applied on the same strip. Hb-A$_2$ estimation was done by elution of the haemoglobin fractions and measurement of absorbance by spectrophotometer at 416 nm wavelength. Hb-A$_2$ > 3.5% was used as the cut-off limit for $\beta$-thalassaemia trait. Serum ferritin was measured by the chemiluminescence method using Access-2 analyzer (Beckman Coulter).

All the collected data were compiled and analyzed in SPSS. The mean values of Hb-A$_2$ and serum ferritin in individuals with and without iron deficiency were compared by the independent t-test, at 5% level of significance. A total of 120 individuals were studied, including 61 females (51%) and 59 males (49%). Their ages ranged from 20 to 50 years. Out of the 120 individuals, 23 (19%) had iron deficiency (serum ferritin < 20ng/ml), whereas the remaining 97 had serum ferritin in the normal range.

In the 23 individuals with iron deficiency, mean Hb-A$_2$ was 4.1 ±0.47% (range 3.0 - 4.8%). Out of these 23
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individuals, 7 were males and 16 were females. In the 97 individuals without iron deficiency, mean Hb-A₂ was 5.1 ±0.58% (range:3.9-6.4%). The difference in the means was statistically significant (p < 0.001). Out of these 23 individuals, who showed iron deficiency, 2 individuals showed Hb-A₂ less than 3.5% (one showed 3.3% and the other 3.0%). One patient showed 3.6%, another had 3.8% and 5 patients showed Hb-A₂ 3.9%.

Serum ferritin in the 59 males ranged from 17-250 ng/ml and in the 61 females it ranged from 10 - 150 ng/ml. The mean ferritin in the 23 iron deficient individuals was 9.4 ng/ml (range 3.3 - 19.6 ng/ml) and in the 97 individuals without iron deficiency it was 67.4 ng/ml (range: 20.5 - 269 ng/ml).

β-thalassaemia is inherited in an autosomal recessive pattern. The prevention programmes against thalassaemia depends upon prenatal diagnosis and screening to identify carriers; having a child of thalassaemia major, can be a source of mental tension and economic burden on the thalassaemia carriers. Iron deficient patients have decreased serum ferritin levels and β-thalassaemia carriers show HbA₂ levels > 3.5%.²

β-thalassaemia carriers have raised HbA₂ level (> 3.5%). However, co-existing β-thalassaemia trait and iron deficiency can mask the expected elevation in Hb-A₂ levels.⁵ This study is unique in studying at the effect of iron deficiency on Hb-A₂ levels in patients with β-thalassaemia trait, confirmed by PCR. The results have clearly shown that the mean level of Hb-A₂ is significantly lower in individuals having β-thalassaemia trait and iron deficiency. Iron deficiency is a far more common problem in Pakistan. Therefore, co-existence of the two problems is not uncommon. This study has shown that patients with β-thalassaemia trait having iron deficiency anaemia could be missed in screening by Hb-A₂ measurement alone. Such individuals can be picked only when PCR for thalassaemia is also included in the screening algorithm. Considering the high cost of PCR, it may be suggested to do PCR for β-thalassaemia in individuals suspected to have co-existing thalassaemia trait and iron deficiency, especially when their spouses are known to have β-thalassaemia traits.

REFERENCES