Role of Estimation of Serum Ferritin, Vitamin B12 and Folic Acid in Management of Beta Thalassemic Children

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Abstract

Objective: To estimate the levels of serum Ferritin, Vitamin B₁₂ and Folic acid in beta thalassemic children.

Introduction: Beta thalassemia is the most common genetically transmitted haematological disorder in Indian children. In thalassemia, there is ineffective erythropoiesis which can be either due to excess iron accumulation, or low vitamin B₁₂ and folate status.

Study Design: The case-control study was carried out at G.G.S. Medical College and Hospital, Faridkot in department of Biochemistry in collaboration with department of Paediatrics.

Materials and methods: In this case-control study, 50 children with with beta thalassemia major in the age group of 4 to 8 years were studied for estimation of serum ferritin, vitamin B₁₂ and folic acid. The control group consisted of 50 age and sex matched healthy children.

Results: The study group had much higher serum ferritin levels as compared to healthy controls (p< 0.001). On other hand, the thalassemic children had much lower vitamin B₁₂ level in comparison with healthy controls (p< 0.001). The folic acid levels were also much lower in thalassemic children as compared to healthy controls (p< 0.001).

Conclusion: Biochemical screening for levels  of serum ferritin, vitamin B₁₂ and folic acid  is of paramount importance in management of thalassemic children. Supplementation of vitamin B₁₂ and folic acid could be helpful to improve erythropoiesis in thalassemic children who have proven deficiency of these vitamins, though further studies are needed to establish this hypothesis.

Key words: Beta thalassemia, Erythropoiesis, Ferritin, Folic acid, Vitamin B₁₂

Introduction

Beta thalassemia is an autosomal recessive genetic disease. The cause is partial or complete lack of ability to synthesise beta chains of the haemoglobin [1]. This process of beta globin chain synthesis is controlled by a gene located on chromosome 11. There can be more than 200 mutations of this gene, leading to varying degrees of inability to synthesise beta chains of haemoglobin. In thalassemia major, there is complete lack of ability to synthesise beta chains of haemoglobin.

This leads to chronic haemolysis and severe anaemia [2]. Patients present with varying degrees of anaemia from early childhood and are transfusion dependent. According to an epidemiological study, beta thalassemia is the most common genetically transmitted haematological disorder in Indian children [3].

Iron metabolism in thalassemia- Normally, the amount of iron (20-30 mg/day) needed for daily production of 300 million new RBCs is provided mostly by the iron which is recycled by macrophages [4]. The iron stored in macrophages is safe, and it does not lead to oxidative stress [5]. Duodenal absorption in normal persons is approximately 1-2 mg/day, which is balanced with iron excretion of 1-2 mg/day. In thalassemic patients, there is increased iron absorption (3-9 mg/day) [6]. This causes increase in body’s iron burden. Additionally in thalassemia major patients, regular blood transfusions lead to double iron accumulation (420 ml of transfused blood is equivalent to 200 mg of iron).

In these patients, the excess iron saturates the plasma transferrin, which then transfers the iron to a storage protein called apoferitin. Thus the storage protein ferritin is formed. Ferritin is a 450 kDa protein...
consisting of 24 subunits which is present in every cell type [7]. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. This fact applies in all types of anaemia of chronic disease due to any cause [8]. In iron overload disorders such as haemochromatosis, serum ferritin levels have been found to be abnormally increased [9].

**Role of vitamin B12 and Folic acid**- Vitamin B\textsubscript{12} and folic acid are essential nutrients for erythropoiesis. Deficiency of either of these vitamins leads to megaloblastic anaemia [10]. Vitamin B\textsubscript{12} deficiency may also cause severe neurological deficit. Low serum folate has well been described in homozygous beta thalassemia [11]. Reports on vitamin B\textsubscript{12} status in thalassemic patients are at variance [12]. In homozygous beta thalassemia, erythropoiesis is depressed. The cause can be iron overload [13] or deficiency of these vitamins.

![Diagram of the role of interdependent cofactor activity of vitamin B12 and folic acid in intracellular DNA synthesis in RBCs.](image)

### Materials and Methods

**Study Design**- The study was carried out at G.G.S. Medical College and Hospital, Faridkot in department of Biochemistry in collaboration with department of Paediatrics. The study was approved by institutional ethical committee of G.G.S. Medical College and Hospital, Faridkot. It was a case-control study. 50 children with with beta thalassemia major in the age group of 4 to 8 years were studied for estimation of serum ferritin, vitamin B\textsubscript{12} and folic acid. The control group consisted of 50 age and sex matched healthy children. Detailed history and complete physical examination was recorded. Informed written consent was taken from all the study subjects.
Exclusion criteria

1. Those on iron or vitamin B-complex supplements.
2. Chronic liver failure as these patients have low vitamin B₁₂ levels.
3. Patients on anti-convulsants and anti-cancer drugs as these patients have low folate levels.
4. Patients with acute infections and acute inflammatory diseases, as these patients show false high ferritin levels. This is because ferritin is an acute phase reactant.
5. Chronic renal failure.

Blood sample collection: About 10 ml of peripheral venous blood was collected from all the study subjects.

Investigations

Routine investigations: These included:

1. Haemoglobin levels and complete blood count. These were done on Beckman Coulter cell counter. Peripheral blood smear was studied for red blood cell morphology.

2. Biochemical investigations included fasting blood glucose, serum electrolytes, liver function tests (SGOT, SGPT, ALP, serum bilirubin) and renal function tests (Blood urea and serum creatinine). The tests were done on Beckman couter AU-480 fully automated analyser. These tests were done to rule out patients of chronic liver failure and chronic renal failure.

Special investigations: These included:

1. Serum ferritin which was measured on Immulite 1000 chemiluminescence. It is based on the principle of solid phase, two site immunometric, chemiluminescence assay. [14] Its normal value in males is 28-365 ng/ml, and its normal value in females is 5-148 ng/ml.[15]

2. Serum vitamin B₁₂ and folic acid. These parameters were measured on Access-2 chemiluminescence machine. Measurement of vitamin B₁₂ and folic acid is based on the principle of competitive binding immunoenzymatic assay. [16] Vitamin B₁₂ or folic acid in the sample binds to the conjugate, preventing the conjugate from binding to the solid phase antibody. After incubation in a reaction vessel, the material bound to the solid phase is held in a magnetic field while the unbound material is washed off. Then the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The photon (light) production is inversely proportional to the concentration of vitamin B₁₂ or folic acid in the sample. Normal value of vitamin B₁₂ is 180-971 pg/ml. [17] And normal value of folic acid is more than 6 ng/ml.[18]

Statistical analysis: Statistical analysis of results was done using SPSS 16 version software. Baseline characteristics of the study subjects were presented as mean + standard deviation. ANOVA (Analysis of Variance) was used for multiple comparisons of parameters between the two groups. p value was calculated to know the significance of difference in the individual variables among the two groups. p value < 0.05 was considered to be statistically significant, while p value < 0.001 was considered to be highly significant. Pearson’s correlation coefficient (r value) was calculated between the values of serum ferritin and serum vitamin B₁₂, and also r value was calculated between the values of serum ferritin and folic acid.

Results

For the present case-control study, subjects were taken after age and sex matching. We compared all the important biochemical parameters between the two groups which included serum ferritin, vitamin B12 and folic acid.

As shown in Table 1, beta thalassemic children had ferritin levels in the range of 2637.4 – 4855.8 ng/ml, while in healthy controls, ferritin levels were in the range of 152.8 – 201.2 ng/ml. (Normal value of ferritin is 28-365 ng/ml in males, and 5-148 ng/ml in females). So ferritin levels were much higher in the beta thalassemic children(Group A) in comparison with the healthy controls(Group B) (p < 0.001).
Table-1: Comparison of serum ferritin, vitamin B\textsubscript{12} and folic acid levels in β-thalassemia patients and healthy controls*

| Biochemical Parameters | Group A (β-thalasemia patients) \n(n=50) | Group B (Healthy controls) \n(n=50) | p value† |
|------------------------|-----------------------------------------------|-----------------------------------------------|----------|
| Ferritin (ng/mL)       | 3746.6 ± 1109.2                              | 177 ± 24.2                                    | 0.0001   |
| Vitamin B\textsubscript{12} (pg/mL) | 245 ± 5.77                                   | 509 ± 112.2                                   | 0.0001   |
| Folic acid (ng/mL)     | 5.02 ± 1.53                                   | 12.2 ± 2.1                                    | 0.0001   |

*The values are expressed in mean ± S.D
† p value < 0.001 means highly significant.

Table-2: Coefficient of correlation between serum ferritin and folic acid, and between serum ferritin and vitamin B\textsubscript{12} in β-thalassemia patients (Group A).

| Biochemical parameters (n=50) | r* | p† |
|-------------------------------|----|----|
| Ferritin and Folic acid      | -0.483 | < 0.001 |
| Ferritin and Vitamin B\textsubscript{12} | -0.724 | < 0.001 |

r value: Pearson’s coefficient of correlation
*Negative r value means negative correlation between the concerned parameters.
† p value < 0.001 means highly significant.

Group A (Beta thalasemic children) had vitamin B\textsubscript{12} levels in the range of 187.3 – 302.7 pg/ml. In comparison, Group B (healthy controls) had serum vitamin B\textsubscript{12} in the range of 396.8 – 621.2 pg/ml. (Normal value of vitamin B\textsubscript{12} is 180 – 971 pg/ml). Hence serum vitamin B\textsubscript{12} levels were significantly lower in beta thalasemic children as compared to healthy controls (p< 0.001).

The levels of serum folic acid in beta thalasemic children (Group A) was in the range of 3.49 – 6.55 ng/ml, while in healthy controls (Group B), serum folic acid levels were in the range of 10.1 – 14.3 ng/ml. (Normal value of serum folic acid is > 6 ng/ml). Hence the levels of serum folic acid were significantly lower in beta thalasemic children as compared to healthy controls (p< 0.001). The p value < 0.001 established significant difference in the levels of all these three parameters between the two groups.

Pearson’s correlation coefficient was calculated between the concerned parameters. In thalasemic patients (Group A), a strong negative correlation was observed between serum ferritin and folic acid (r = -0.483, p < 0.001), indicating high level of significance.

Also, there was strong negative correlation between serum ferritin and vitamin B\textsubscript{12} levels in these study subjects (r = -0.724, p < 0.001). Hence with increasing levels of serum ferritin, there was corresponding decrease in the levels of serum vitamin B\textsubscript{12} and folic acid.

Scatter plots representing correlation between serum ferritin and vitamin B\textsubscript{12}, and between serum ferritin and folic acid in β-thalassemia patients have been shown in Figure 1 and 2 respectively.
As can be seen from the above figure, with an increase in serum ferritin levels, there was a corresponding decrease in the levels of serum vitamin B\textsubscript{12} in beta thalassemic children. Pearson’s correlation coefficient (r value of -0.7245) indicates strong negative correlation between serum ferritin and serum vitamin B\textsubscript{12} levels.

As can be seen from the above figure, with an increase in serum ferritin levels, there was a corresponding decrease in the levels of serum folic acid in beta thalassemic children. Pearson’s correlation coefficient (r value of -0.4836) indicates strong negative correlation between serum ferritin and serum folic acid levels.
Discussion

Anaemia affects a significant proportion of children in developing countries. Nutritional deficiencies like iron, vitamin B$_{12}$ and folic acid are also very frequent in these areas [19]. So for treatment of anaemia, we need detailed evaluation to find out the exact cause.

In our study, thalassemic patients had much higher ferritin levels in comparison with healthy controls. Their PBF picture showed microcytic hypochromic picture. Microcytosis may lead to confusion with iron deficient state. So these patients sometimes receive costly and unnecessary iron treatment. [20] Hence knowing iron levels is of utmost importance in these patients to avoid harmful effects of iron overload in early stages of the disorder [21]. On other hand in iron deficiency anaemia, ferritin levels are decreased and these patients need iron treatment.

In our present study, children with beta thalassemia were found to have significantly lower vitamin B$_{12}$ and folic acid levels as compared to healthy controls (p<0.001, p< 0.001 respectively). The healthy controls had normal vitamin B$_{12}$ levels (180-971pg/ml)[17], as well as normal levels of folic acid (>6 ng/ml)[18].

According to a study by Crayn et al, the most important PBF findings of vitamin B$_{12}$ and folic acid deficiency are macrocytic RBCs and hypersegmented neutrophils [22]. This increase in RBCs size may be masked by microcytosis of co-existing iron deficiency or thalassemia. So normal MCV levels may be seen in up to one-thirds of the patients with vitamin B$_{12}$ deficiency[23]. Hence haematological picture alone in these thalassemic patients cannot provide complete insight to guide their treatment.

Our study illustrated strong negative correlation between serum ferritin and vitamin B$_{12}$ levels in the thalassemic study group (r= -0.72, p< 0.001 ), as shown in Figure 2. The negative relationship between serum ferritin and vitamin B$_{12}$ may be due to increased synthesis of HbA2 in thalassemic patients.[24] A study by Tamagnini GP et al also illustrated vitamin B$_{12}$ deficiency in patients of beta thalassemia [25].

Our study showed strong negative correlation between serum ferritin levels and serum folic acid levels in the thalassemic study group(r= -0.483, p<0.001), as shown in Figure 3. A study by Silva A et al with similar findings support our results [26]. Another study demonstrated salutary effect of folate administration in beta thalassemic patients [27].

Hence since vitamin B$_{12}$ and folic acid facilitate normal erythropoiesis, keeping their blood levels normal is very essential in beta thalassemic patients.

Key message and Conclusions- Biochemical screening for the levels of serum ferritin, folic acid and vitamin B$_{12}$ is of utmost importance in beta thalassemic patients of paediatric age group. The increased serum ferritin, and decreased serum vitamin B$_{12}$ and folate add to ineffective erythropoiesis in beta thalassemic patients.

Their PBF picture of microcytosis may lead to confusion with iron deficiency anaemia.

Hence these anaemic children should be thoroughly investigated so that proper treatment is instituted to avoid the cost and side-effects of unnecessary medications like iron therapy. Our study could be beneficial for thalassemic patients as vitamin B supplementation can be useful in these patients who have proven deficiency of these vitamins, though further studies are required to prove the hypothesis.

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