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To study the impact of iron treatment on hemoglobin A2 in iron deficient B Thalassemia minor cases in a tertiary care hospital of central India: a Cross Sectional Study

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Abstract

Introduction: Thalassemia syndromes and iron deficiency anaemia (IDA) are the two most commonaetiologies of microcytic hypochromic anaemia in children and adults. It has long been considered that iron deficiency does not exist in thalassemia syndromes, including thalassemia major as well as trait andvery few studies has been carried out till date.

Objectives: 1.To estimate prevalence of iron deficiency anaemia & hematological parameters with serum iron studies in -thalassemia minor patients 2.To analyse haematological β and haemoglobin A2 parameters of iron deficiency anaemia among β-thalassemia minor patients before and after iron therapy.

Materials and Method: It was a Cross sectional study conducted in HPLC diagnosed beta-thalassemiaminor patients over a period of 2 year at tertiary care hospital of Nagpur. A semi structured questionnaire was designed and ethical clearance was obtained.

Results: The prevalence of concomitant IDA with β-thalassemia minor was found to be 43.33%. Individuals with iron deficiency anemia reveals that the mean S. Iron was 42.88 *μg/dl* and mean serum ferritin was 7.87 *ng/dl* ± 1.84. After the 3 month iron therapy the mean serum iron was increased significantly to 74.03 *μg/dl* and serum ferritin increased significantly to 48.21± *5.92*. Similarly, the mean TIBC was 507.27 *μg/dl* ± 34.68 before iron therapy. It decreased to 367.91 *μg/dl* ± 16.62 after the 3 month iron therapy. These differences were found to be statistically significant.

Conclusions: Simple investigations like iron profile should be used in patients with borderline HbA2 or otherwise suspected thalassemia minor cases in order to identify IDA. HbA2 value should be reassessed after treatment of IDA to confirm the diagnosis of thalassemia minor.

Keywords: iron deficiency anaemia, β-thalassemia minor, prevalence, haemoglobin A2.

Introduction

The estimated prevalence of B thalassemia in different regions of India is reported to vary between 2.7 and 14.9%, with an average of 4.5%.[1,2] Approximately 5000–6000 children with homozygous β- thalassemia are born annually.[2] To prevent the birth of a child with thalassemia major, detection of the heterozygous state is critical.

Elevation of the level of HbA2 is specific for the diagnosis of a β-thalassemia trait.[3-5] In India, deficiency of iron is also widely prevalent. [6] Both disorders are characterized by the presence of microcytic hypochromic red cells. [7] It has been observed that in populations where iron deficiency is common, the heterozygous state of β-thalassemia may be missed due to a reduction in the level of HbA2.

[3,8] However, studies from India have shown that HbA2 is not significantly lowered in the presence of iron deficiency.[9] Similar results have been reported from a study of British Asian children.[10] Thalassemia syndromes and iron deficiency anaemia (IDA) are the two most common aetiologies of microcytic hypochromic anaemia in children and adults. It has long been considered that iron deficiency does not exist in thalassemia syndromes, including thalassemia major as well as trait. However, studies have shown the occurrence of iron deficiency in patients with beta thalassemia trait (BTT). Earlier authors have demonstrated lower initial haemoglobin levels in patients with coexisting IDA and BTT. [11-13] This has been explained by the lack of hematopoietic nutrients due to iron deficiency superimposing on the imbalance in globin chain synthesis.[21] Similar changes have also been shown in other red cell parameters, serum iron, ferritin, and total iron binding capacity. These changes have also been demonstrated to improve after adequate iron replacement therapy.[11, 12,15] HbA2 levels have been reported to be lower in patients with coexisting IDA and BTT, with improvement in levels after iron therapy. [03, 11] However, other studies have shown no significant difference in HbA2 levels in such patients. [16, 17] The reduction in HbA2 levels in patients with concomitant BTT and IDA has been suggested to interfere in the diagnosis of the former. A recent study has hypothesized that such an occurrence can lead to these patients with BTT marrying another person with BTT with increased risk of birth of thalassemia major child.[18] An extensive search of the available literature yielded only few Indian reports of concomitant BTT and iron deficiency anaemia.[14, 19-21] None of these studies evaluated the effect of iron therapy on red cell parameters, iron status, and haemoglobin subtypes in Indian BTT patients with concomitant iron deficiency. So the present study was carried with following aim & objectives.

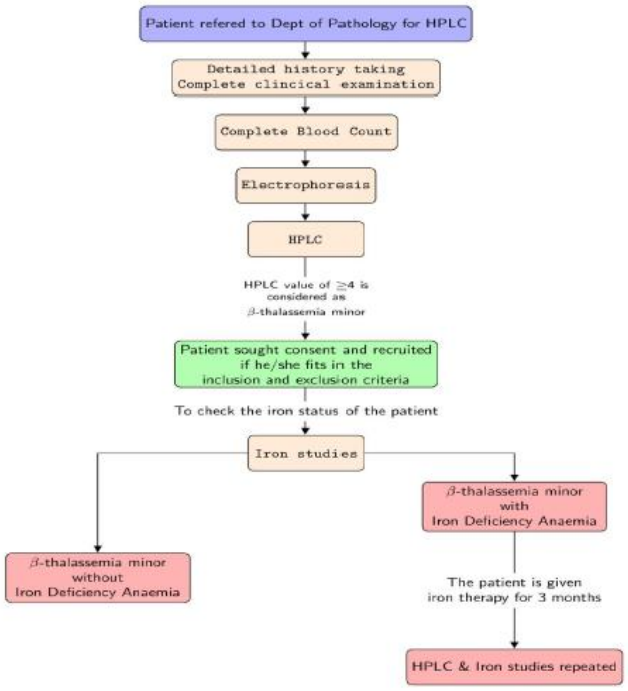
Aim and Objectives

1. To estimate hematological parameters with serum iron studies in -thalassemia β minor patients.
2. To estimate the prevalence of iron deficiency anaemia in β-thalassemia minor patients.
3. To analyse haematological and haemoglobin A2 parameters of iron deficiency anaemia among β-thalassemia minor patients before and after iron therapy.

Materials and Methods

The present Cross sectional study was conducted in beta-thalassemia minor patients over a period of 2 years in a tertiary care hospital of central India. A total of 60 HPLC diagnosed beta-thalassemia minor patients were included in study. Patients having other haemoglobinopathies, thalassemia major, migrant population (due to poor follow-up) & patients who have received blood transfusion within the last 3 months were excluded. Complete enumeration of b thalassemia minor patients attending tertiary care hospital was done. The sample size for a given study was 60. Non probability Convenience sampling method was used for the selection of participants. A semi structured questionnaire was designed based on the aim and objectives of the study and after reviewing of literature. Detailed history of the patients was taken and complete clinical examination was done. After the consent from the patients, 5ml of the patient's blood was collected, 2ml was collected in 4% K2 EDTA (ethylene diamine tetra-acetic acid) anticoagulant bulb for

haematological work-up and rest of the sample was collected in plain bulb for serum iron studies. So, in this study thalassemia minor cases having HbA2 level >4 were considered and seen for iron deficiency. The iron deficiency thalassemia minor patients were given oral iron therapy of Tab. Ferrous Sulphate 200mg once daily for 3 months. Three months later, all the laboratory investigations were performed again and the results were compared with the baseline investigations to see the effect of iron therapy on the thalassemia minor cases. Ethical clearance was obtained from the Institutional Review Board of the College after submitting the protocol & subsequently answering all their queries. Then the final data collection was initiated



Statistical Analysis

The data was recorded into an Excel Spread sheet after collection and was then analysed using statistical software Epi Info 7.1.5.0. Descriptive statistics like frequency and proportion while continuous variables were reported using mean and standard deviations. Associations between the variables were reported using Chi squared ($\chi 2$) test for categorical variables & Paired t-test for continuous variables.

Results

Table 1**:** Distribution of the study population according to Age Gender & religion (N=60)

Parameter	Description	Frequency	Percentage
Age group	10 years	4	6.67%
	11-30 years	13	21.67%
	31-50 years	35	58.33%
	51-70 years	5	8.33%
	>70 years	3	5.00%
Gender	Male	21	35%
	Female	39	65%
Religion	Buddhist	27	45%
	Hindu	22	36.67%
	Muslim	11	18.33%

In the present study, the mean age of the study population was found to be 36.45 ±17.04 years. The youngest study subject aged 3 years and the oldest aged 76 years, with a range of 73 years. Majority of the study population were women (65%). Men consisted only of 35% of the study population. Buddhists consisted of the majority of the study population (45%); followed by Hindus (36.67%) and Muslims (11%). (Table1)

Table 2: Hematological Parameters and Iron studies of the study subjects (N=60)

Parameter	Mean	Std. Dev	Min	Max
Hb (%)	10.46	1.46	7.70	12.80
RBC (million)	4.54	0.58	3.50	5.50
PCV (%)	39.01	9.28	20.50	55.00
MCV (fL)	65.90	7.88	52.00	79.00
MCH (pg)	20.43	2.45	12.00	25.00
MCHC (g/dl)	28.27	3.54	20.00	33.00
RDW (%)	14.44	1.85	11.10	17.00
PLT (lakh/mm^3)	2.89	0.87	1.60	4.40
WBC(/mm^3)	7274.17	2037.79	4130	10940
HbA2 (%)	5.51	0.79	4.10	7.00

The haematological parameters are summarised in Table 2. The mean haemoglobin was found to be 10.46%± 1.46%, RBC: 4.54 ±0.58, PCV: 39.01%±9.28%, MCV: 65.90 f l. ± 7.88 f l., MCH: 20.43 pg ±2.45 pg, MCHC:

28.27g/dl± 3.54 g/dl, RDW: 14.44% ±1.85%, Platelets: 2.89 lakh/mm3 ±0.87 lakh/mm3 and the mean WBC count was found to be 7274.17 ±2037.79. The mean HbA2% was found to be 5.51 with a standard deviation of 0.79. The HbA2 values ranged from 4.10 – 7.00.

Table 3: Distribution of age (in years) and gender according to iron deficiency anemia status

IDA Status	Female	Male	Total (%)	Mean age	Std. Dev
β-thalassemia minor with IDA	18	08	26 (43.33)	39.7	17.7
β-thalassemia minor without IDA	21	13	34 (56.67)	33.9	16.3

Total 26 individuals were diagnosed with having -thalassemia minor and concomitant β iron deficiency anemia (IDA). The prevalence of concomitant IDA with β-thalassemia minor was found to be 43.33%. Majority were female (18/26). Similarly there were more women than men without iron deficiency anemia (21/34). However, this difference was not found to be statistically significant. (Table3)

Table 4: Iron Profile of the study subjects with and without iron therapy deficiency anemia.

Parameter	Treatment Status	Mean	Std. Dev	t-statistic	p-value
S. Iron (*mg*/dl)	With IDA	42.88	7.26	17.65	<0.001
	Without IDA	72.88	5.41		
	Overall	59.88	16.23		
S. TIBC (*mg*/dl)	With IDA	507.27	34.68	16.86	<0.001
	Without IDA	373.22	24.02		
	Overall	431.31	72.93		
S. Ferritin (ng/dl)	With IDA	7.87	1.84	36.97	<0.001
	Without IDA	48.66	6.08		
	Overall	30.98	10.92		

The present study revealed that the mean serum iron and mean serum ferritin were significantly lower among individuals with IDA as compared to those that did not have concomitant IDA with β-thalassemia minor (p-value <00.001. The mean S. TIBC was 431.31 *mg*/dl ±72.93 *mg*/dl among the study subjects. It was found that the mean serum TIBC was significantly higher among individuals with IDA as compared to those that did not have concomitant IDA with β-thalassemia minor (p-value <0.001). (Table 4)

Table 5. Hematological Parameters of the concomitant IDA with β-thalassemia minor subjects before and after iron therapy (N=26)

Parameter	Treatment Status	Mean	Std. Dev	t-statistic	p-value
Hb%	Before Treatment	9.04	0.70	-9.222	<0.001
	After Treatment	10.57	0.98		
RBC (million)	Before Treatment	4.64	0.57	0.93549	0.3585
	After Treatment	4.48	0.64		
PCV (%)	Before Treatment	40.90	10.37	-7.8837	<0.001
	After Treatment	43.68	10.19		
MCV (fl.)	Before Treatment	57.96	2.63	-29.009	<0.001
	After Treatment	65.92	2.74		
MCH (pg)	Before Treatment	19.38	3.01	-7.8633	<0.001
	After Treatment	20.77	3.13		
MCHC (g/dl)	Before Treatment	24.62	1.90	-8.3799	<0.001
	After Treatment	26.23	2.41		
RDW (%)	Before Treatment	16.15	0.73	4.8176	<0.001
	After Treatment	14.88	1.45		
PLT (lakh/mm^3)	Before Treatment	2.85	0.83	-1.0017	0.3261
	After Treatment	3.05	0.79		
WBC(/mm^3)	Before Treatment	7228.46	2110.56	-1.5761	0.1276
	After Treatment	7998.08	2003.43		

All the haematological parameters were improved after iron therapy significantly except RBC count. (Table 5)

Table 6: Iron Profile & HbA2% of the concomitant IDA with -thalassemia minor β subjects before and after iron therapy (N=26).

Parameter	Treatment Status	Mean	Std. Dev	t-statistic	p-value
S. Iron (*mg*/dl)	Pre-treatment	42.88	7.26	-16.858	<0.001
	Post-treatment	74.03	6.00		
S. TIBC (*mg*/dl)	Pre-treatment	507.27	34.68	18.479	<0.001
	Post-treatment	367.91	16.62		
S. Ferritin (ng/dl)	Pre-treatment	7.87	1.84	-33.169	<0.001
	Post-treatment	48.21	5.92		
HbA2%	Pre-treatment	4.1	0.77	-2.0728	0.04865
	Post-treatment	4.5	0.87		

Table no 6 shows that mean S. Iron was 42.88 *μg/dl* and mean serum ferritin was 7.87 *ng/dl* ± 1.84 in Individuals with iron deficiency anemia. After the 3 month iron therapy the mean serum iron was increased significantly to 74.03 *μg/dl* and serum ferritin increased significantly to 48.21± *5.92*. Similarly, the mean TIBC was 507.27 *μg/dl* ± 34.68 before iron therapy. It decreased to 367.91 *μg/dl* ± 16.62 after the 3 month iron therapy. These differences were found to be statistically significant. The effect of treatment on the HbA2 can be depicted by the increase in HbA2 values before and after the treatment. The mean HbA2 after the 3 month iron therapy was significantly higher than before treatment (p-value = 0.04865) implying that the treatment was effective.

Discussion

Iron deficiency anaemia and thalassemia syndromes, especially -thalassemia β minor, are the two most frequent microcytic hypochromic anaemias highly prevalent in countries like India.[27, 28] The National Family Health Survey (NFHS-3) of 2011 reveals the prevalence of anaemia as 70–80% in children, 70% in pregnant women, and 24% in adult men. The prevalence of *b*-thalassemia minor has been cited as 3.5– 10% in India.[29] In present study, mean hemoglobin was found to be 10.46% ± 1.46%, RBC: 4.54 ± 0.58, PCV: 39.01% ± 9.28%, MCV: 65.90 *f l*. ± 7.88 *f l*., MCH: 20.43 *pg* ± 2.45 *pg*, MCHC: 28.27*g/dl* ± 3.54 *g/dl*, RDW: 14.44% ± 1.85%, Platelets: 2.89 lakh/mm3 ± 0.87 lakh/mm3 and the mean WBC count was found to be 7274.17 ± 2037.79. The mean HbA2% was found to be 5.51 with a standard deviation of 0.79. The HbA2 values ranged from 4.10 – 7.00. **Rahman *et. al.* (2015).**[24] found that among patient with β thalassemia minor the mean Hb was 10.23 ± 147, mean MCV was 72.05 ± 5.24, mean MCHC was 21.65 ± 2.69. **Arshad *et. al.* (2016).** [25] found that mean HbA2 in withβthalassemia minor was 5.1 ± 0.58. **Sharma *et. al.* (2015)** .[23] performed a case-control study in Dept. of Hematology, PGIMER Chandigarh and found that Mean HbA2 in β-thalassemia minor was 5.4 ± 0.8 (range 3.1–7.9). In present study total 26 individuals were diagnosed with having *β-thalassemia* minor and concomitant iron deficiency anemia (IDA). The prevalence of concomitant IDA with *β-thalassemia* minor was found to be 43.33%. The mean serum iron of the 26 individuals diagnosed with concomitant IDA with *β-thalassemia* minor was found to be 42.88 ± 7.26 *μg/dl*.The mean serum ferritin among the individuals with IDA was found to be 7.87 ± 1.84. The mean serum TIBC was found to be 507.2+ 37 ± 34.68. Similar results found in previous studies, as **Kamal *et. al.* (2015).** [22] conducted a study in northeastern Iraq and found that all hematological parameters were significantly lower in *β-thalassemia* minor and coincident iron deficiency with *β-thalassemia* minor in comparison to the control group. **Rahman *et. al.* (2015).** [24] found that prevalence of iron deficiency anemia was found to be 30.2% among *β-thalassemia* trait.

In present study individuals with iron deficiency anemia reveals that the mean S. Iron was 42.88 *μg/dl* pre-treatment. After the 3 month iron therapy the mean serum iron was increased to 74.03 *μg/dl*. This difference was found to be statistically highly significant. The mean serum ferritin was found to be 7.87 *ng/dl* ± 1.84 among the 24 individuals diagnosed with concurrent iron deficiency anemia and β- thalassemia minor. It is found that serum ferritin increased significantly after 3 months of therapy.

Similarly, the mean TIBC was found to be 507.27 *μg/dl* ± 34.68 before iron therapy. It decreased to 367.91 *μg/dl* ± 16.62 after the 3 month iron therapy. This difference was found to be statistically significant. There were no studies on treatment of iron deficiency anemia in β thalassemia trait. In our study it is found that there was significant improvement in hematological parameters of β Thalassemia trait patients with iron deficiency anemia

after treatment. We used serum ferritin levels <15 ng/mL as a cut off for iron deficiency, as in previous studies. [12, 13, 26]. **Saraya et al.** reported that low serum ferritin was more frequently seen in female patients with BTT than male patients and recommended iron supplementation for females.[14] Serum ferritin levels improved significantly and reached normal values after iron therapy. These results are similar to previous studies. [15]. In present study mean HbA2 value before the treatment was 4.1% ± 0.77 and after treatment it was 4.5% ± 0.87. The effect of treatment on the HbA2 can be depicted by the increase in HbA2 values after the treatment. The mean HbA2 after the 3 month iron therapy was significantly higher than before treatment implying that the treatment was effective. HbA2 levels, in our patients, improved significantly (p-value < 0.001) after iron replacement therapy. **Wasi et al, El-Agouza et al ,Keramati et al** have been reported similar results as in our study .[3, 15, 19] These findings underscore the importance of treating iron deficiency for a minimum of 12-16 weeks, especially if HbA2 levels are borderline.

Conclusion

The present study highlights the coexistence of iron deficiency anaemia in beta thalassemia minor patients.

- The diagnosis of beta thalassemia minor can be confounded due to low level of HbA2 in concomitant Iron Deficiency Anaemia (IDA) and thalassemia minor.
- Screening of thalassemia minor in our study population with a high prevalence of iron deficiency does not preclude the thalassemia minor in majority of carriers despite IDA's predilection to lower the HbA2.
- In a patient with a strong suspicion of thalassemia minor (family history of thalassemia and borderline range of HbA2 values) iron deficiency should be identified with simple laboratory investigations like iron profile.
- Iron deficiency can be corrected with appropriate treatment.
- The outcome of present study and available evidences recommend the utility of simple investigations like iron profile in the case of patients with borderline HbA2 or otherwise suspected thalassemia minor in order to identify IDA and not miss patients of thalassemia minor.
- HbA2 value should be reassessed after treatment of IDA to confirm the diagnosis of Thalassemia minor.

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