Effect of iron therapy on red cell indices and hemoglobin subtypes on patients with beta-thalassemia trait who developed iron-deficiency anemia: a tertiary center experience

Sawsan M. Moeen[a], Ahmad F. Thabet[a], Marwa M. Thabet[b]

[a]Department of Internal Medicine, Clinical Hematology Unit, [b]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence to Dr. Sawsan Mohamed Moeen, MD Clinical Hematology, Faculty of Medicine, Assiut University, Assiut, Egypt, Postal code 71515. Tel: +201005475441; fax: +20882333327; e-mail: sawsan.moeen@yahoo.com

Received 6 March 2019
Accepted 13 June 2019
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:741–745

Background
The combination of beta-thalassemia trait (BTT) and iron-deficiency anemia (IDA) is an interesting common issue in our country. However, treatment of this condition is challenging.
Objectives
This prospective observational study was designed to evaluate the effect of oral iron therapy on red cell indices, iron status, and hemoglobin A_2 levels in patients with BTT who developed IDA.
Patients and methods
A total of 50 patients with BTT who developed IDA were included. A complete blood count, iron status, and follow-up hemoglobin electrophoresis by high-performance liquid chromatography were done. The patients with BTT received oral iron therapy of 60 mg elemental iron three times/day for a period of 5 months, and the investigations were repeated after 3 and 5 months of treatment.
Results
There was a statistically significant increase in hemoglobin level ($P<0.001$ each), mean corpuscular volume ($P<0.001$ each), mean corpuscular hemoglobin ($P=0.004$ and $P<0.001$, respectively), mean corpuscular hemoglobin concentration ($P=0.007$ and $P=0.001$, respectively), serum iron ($P<0.001$ each), serum ferritin ($P<0.001$ each), and hemoglobin A_2 levels ($P=0.001$ and $P=0.005$, respectively), whereas significant decrease in the total iron-binding capacity ($P=0.001$, and $P=0.005$, respectively) after the third and fifth month of oral iron therapy.
Conclusion
It is important to suspect, recognize, and correct IDA in patients with BTT and to repeat hemoglobin electrophoresis after iron therapy.

Keywords:
anemia, beta-thalassemia trait, iron deficiency, serum ferritin

Egypt J Intern Med 31:741–745

1110-7782

Introduction

Iron-deficiency anemia (IDA) and beta-thalassemia are the most common causes of microcytic hypochromic anemia [1,2]. In some cases, it is challenging to distinguish IDA from hemoglobinopathy. On the contrary, patients diagnosed with beta-thalassemia trait (BTT) can develop iron deficiency with the subsequent need for iron therapy [3]. However, some studies reported lower hemoglobin levels in patients with coexisting IDA and BTT [1]. Diagnosis of BTT depends on increased levels of hemoglobin A_2 (HbA_2), which is found at low levels (<3%) in normal healthy individuals and elevated levels (≥3.5%) in BTT [4]. The classical phenotype of heterozygous beta-thalassemia includes high HbA_2 level and red cell count a markedly decreased mean corpuscular volume (MCV) (60–75 fl) and mean corpuscular hemoglobin (MCH) levels (18–24 pg) [5]. Deficiency of iron can modulates the synthesis of HbA_2 resulting in decreased HbA_2 levels [6], making the diagnosis of BTT more challenging. Heterozygous beta-thalassemia is usually silent clinically. So, accurate detection of the carrier is important [5]. Iron deficiency results in changes in red blood cell indices, serum iron, ferritin, and total iron-binding capacity (TIBC) [7]. Moreover, IDA may decrease HbA_2 levels and interfere with diagnosis of BTT. There are few studies, about concomitant BTT and iron deficiency [8,9]. Some of these studies have assessed the effect of iron therapy on hemoglobin subtypes and iron status [10].

The aim of the present study was to evaluate the effect of treatment with oral iron therapy of 60 mg elemental

iron three times/day for a period of 5 months on red blood cell indices, iron status, and HbA_2 and HbF levels in patients diagnosed as having BTT who developed IDA.

Patients and methods

This prospective observational study included 50 patients diagnosed with BTT who developed IDA (23 females and 27 males). Participants were included in the study if their HbA_2 level more than 3.7%, hemoglobin level less than 10 g/dl, low serum ferritin levels less than 12 ng/ml, no evidence of other hemoglobinopathy, and normal random blood sugar levels. Cases with other hemoglobinopathies, have normal or high ferritin level, and refuse to participate in the study were excluded. Patients were invited to participate in the study after explaining objectives and steps of the research to them. The study was approved by the Committee of Medical Ethics of the Faculty of Medicine. Written consents were obtained from all participants. The study patients were recruited from Clinical Hematology Unit, Internal Medicine Department and Health Insurance, from July 2017 till September 2018.

All patients were subjected to full history taking including age, sex, with special stress on nutritional history and family history of thalassemia; thorough clinical examination, with careful assessment of clinical signs relevant to thalassemia such as pallor, jaundice, and splenomegaly; and laboratory assessments.

For all participants, laboratory investigations including complete blood count, serum iron, TIBC, serum ferritin, and hemoglobin electrophoresis were done.

An automated cell counter (ADVIA 120 Hematology System, Siemens, Germany) was used to perform complete blood count. Serum iron and TIBC were determined calorimetrically, and ferritin was measured turbidimetrically by using Hitachi-911 Automatic Analyzer (Boehringer Manheim, Tokyo, Japan) and commercially available kit. Hemoglobin electrophoresis was done by high-performance liquid chromatography by D10 system, where 5 µl of blood was withdrawn and hemolyzed before injection in 1-ml buffer. The analytes are eluated at a flow rate of 2 m/min. There are established windows with specific retention times for the most frequently occurring hemoglobin, and the results of patients were interpreted according to it.

Participants received an oral iron therapy 60 mg three times/day for a period of 5 months, and all laboratory investigations were repeated after 3 and 5 months of oral iron therapy.

Statistical analysis

Data entry and analysis were performed using a statistical software package SPSS. *P* value less than 0.05 was considered statistically significant. Descriptive statistics as mean and SD were calculated. Test of significance as paired *t* test was used to compare the mean difference before and after therapy.

Results

This study included 50 patients with BTT who developed IDA (23 females and 27 males). Their mean age was 17.48±0.89 years, body weight was 47.72±3.76 kg, and body height was 156.14±4.29 cm. The baseline demographic and laboratory parameters of the studied patients with BTT who developed IDA are shown in Table 1.

In the present study, we found a statistically significant increase in mean levels of hemoglobin ($P<0.001$), red blood cell indices [MCV ($P<0.001$ each), MCH ($P=0.004$ and $P<0.001$, respectively), and mean corpuscular hemoglobin concentration (MCHC) ($P=0.007$ and $P=0.001$, respectively)], serum iron ($P<0.001$ each), and serum ferritin ($P<0.001$ each), in patients with BTT who developed IDA after 3 and 5 months of oral iron therapy.

Interestingly, mean HbA_2 levels were significantly increased after 3 and 5 months of oral iron when compared with their baseline ($P=0.001$, $P=0.005$, respectively). However, there was no significant difference between HbF levels before and after iron therapy. However, a statistically significant decrease of the mean levels of TIBC ($P<0.001$) and red cell distribution width ($P=0.004$, $P=0.009$) was observed after 3 and 5 months of treatment (Table 2, Fig. 1a–c).

Oral iron therapy was well tolerated with minimal adverse effects, such as abdominal pain and

Table 1 Baseline demographic and laboratory data of the studied 50 patients with beta-thalassemia trait

Variables	Patients with BTT with IDA (*N*=50) (mean ±SD)
Age (years)	17.48±0.89
Body weight (kg)	47.72±3.76
Body height (cm)	156.14±4.29
Hemoglobin (g/dl)	8.96±0.73

BTT, beta-thalassemia trait; IDA, iron-deficiency anemia.

Table 2 Follow-up of red cell indices, iron status, and hemoglobin A_2 levels among studied 50 patients with beta-thalassemia trait after 3 and 5 months of oral iron therapy

Variables	Before treatment (*N*=50) (mean±SD)	After 3 months of oral iron therapy (*N*=50) (mean±SD)	*P* value[a]	After 5 months of oral iron therapy (*N*=50) (mean±SD)	*P* value[b]
Hemoglobin (g/dl)	8.96±0.73	11.03±0.34	<0.001	13.55±0.79	<0.001
MCV (fl)	65.0±1.86	68.52±1.99	<0.001	70.16±2.02	<0.001
MCH (pg)	17.80±1.16	18.20±0.83	0.004	20.38±1.35	<0.001
MCHC (g/dl)	29.96±1.37	30.74±1.41	0.007	32.02±1.58	0.001
RDW %	19.76±3.34	18.68±2.96	0.004	17.24±3.43	0.009
Serum iron (μg/dl)	24.04±2.05	70.96±3.92	<0.001	84.14±9.54	<0.001
TIBC (μg/dl)	421.98±9.06	353.9±11.72	<0.001	293.76±21.76	<0.001
Serum ferritin (ng/ml)	8.46±1.01	14.02±1.69	<0.001	56.78±10.43	<0.001
HbA_2 (%)	5.07±0.44	5.16±0.43	0.001	5.20±0.39	0.005
HbF (%)	1.59±0.62	1.48±0.59	0.112	1.43±0.57	0.697

Hb, hemoglobin; HbA_2, hemoglobin A_2; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; TIBC, total iron-binding capacity. [a]*P* value=difference between baseline and after 3 months of treatment. [b]*P* value=difference between 3 and 5 months of treatment. Paired *t* test was used. *P* value less than 0.05 was considered significant.

constipation in four (8%) patients and occasional nausea in six (12%) patients, but these adverse effects were tolerated by the patients.

Discussion

IDA and thalassemia traits are common causes of hypochromic microcytic anemia that a clinician faces in the blood picture, so it is important for a hematologist to differentiate between both conditions [11]. Interestingly, both IDA and BTT may present in the same individual, and such cases should be identified and treated properly to improve and recover their anemia [12].

The morphological findings in both IDA and BTT are so close that it is really hard to differentiate [13]. Thus, prompt measures should be taken to prevent and treat anemia in those patients to reduce associated morbidity [14] and to have better quality of life.

Understanding the role of thalassemia trait in iron balance and metabolism is still a hot area need to be explored. Moreover, appropriate evaluation of iron status in patients with BTT is needed [1].

Some studies have reported lower levels of hemoglobin and HbA_2 in patients with concomitant IDA and BTT, which improved after oral iron treatment [1,15], whereas other studies found no significant difference in HbA_2 levels in these patients [16].

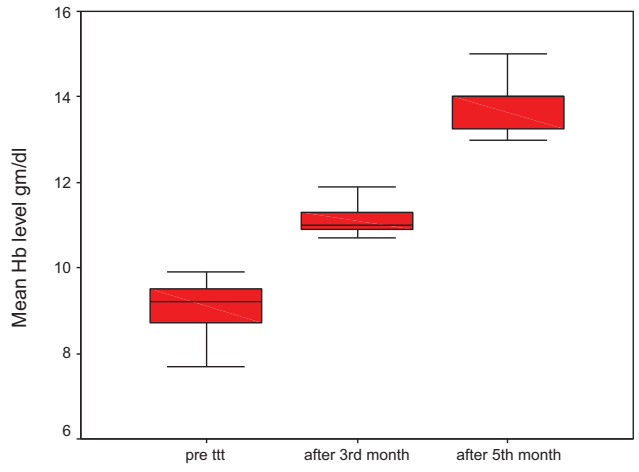
In the present study, we reported a statistically significant increased mean levels of hemoglobin ($P<0.001$ each), red blood cell indices [MCV ($P<0.001$ each), MCH ($P=0.004$ and $P<0.001$, respectively), MCHC ($P=0.007$ and $P=0.001$, respectively);; serum iron ($P<0.001$ each), and serum ferritin ($P<0.001$ each) in patients diagnosed with BTT who developed IDA after 3 and 5 months of oral iron therapy.

Moreover, in this study, we reported a significant elevation of mean HbA_2 levels after 3 and 5 months of oral iron therapy ($P=0.001$ and $P=0.005$, respectively). However, there was no statistically significant difference in HbF levels between before and after oral iron therapy ($P=0.112$ and $P=0.697$, respectively).

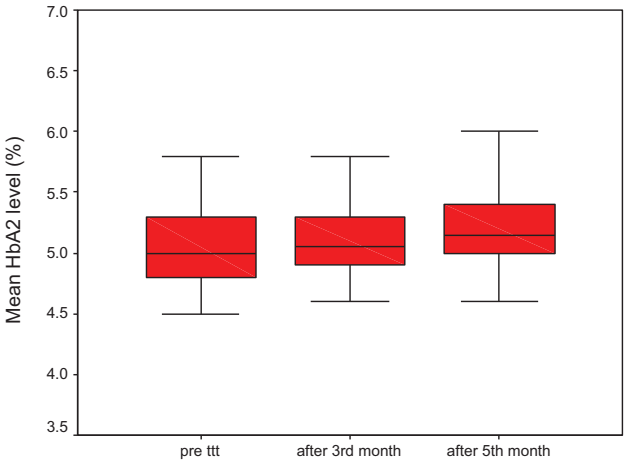
These results are in concordance with the study by Verma *et al.* [10], who found a significant increased mean levels of hemoglobin of the studied 20 patients with BTT with concomitant IDA after oral iron therapy for a period of 20 weeks ($P<0.001$). Additionally, increased mean levels of MCV ($P=0.01$), MCH ($P=0.04$), MCHC ($P=0.04$), serum iron ($P<0.001$), and serum ferritin ($P<0.001$) were noted, and they added that assessment of iron status in patients with BTT becomes more interesting, as formerly iron overload had been found in patients with thalassemia major, but lately iron deficiency was found in patients with BTT.

Our results are in similar to those of Dolai *et al.* [1], who assessed iron levels among Indian patients with BTT ($n=150$). Of 150 patients with BTT, 29 patients had IDA. Those patients with BTT who had iron deficiency had lower levels of hemoglobin (9.78±0.19) and MCH (20.18±0.4) compared with those patients

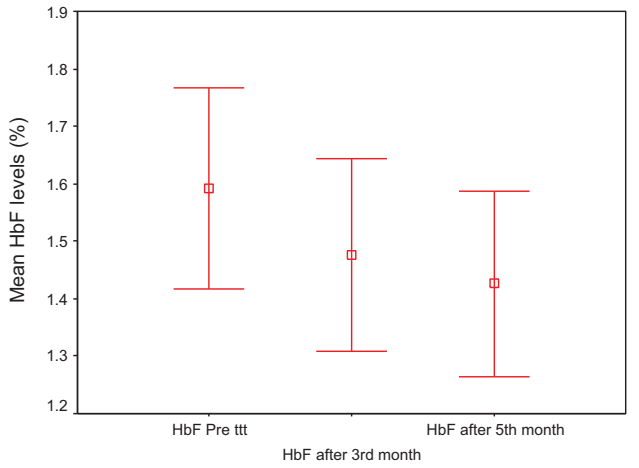
Figure 1



A Follow-up of Hb levels after 3rd and 5th months of iron therapy



B Follow-up of HbA2 level after 3rd and 5th months of iron therapy



C Follow-up of HbF levels after 3rd and 5th months of iron therapy

(a) Follow-up of Hb levels of the studied patients with BTT. (b) Follow-up of HbA_2 levels of the studied patients with BTT. (c) Follow-up of HbF levels of the studied patients with BTT. BTT, beta-thalassemia trait; HbA_2, hemoglobin A_2.

without IDA ($P<0.0$, for each). Hence, they concluded that iron deficiency is a common existing condition in BTTs. Moreover, the study suggested that assessment of iron level among patients with BTT should be done and iron deficiency if detected should be treated. Patients with BTT have severe IDA, and if recognized and treated, they will have better prognosis. Moreover, Saraya *et al.* [17] found iron deficiency in patients with BTT, and they suggested that, iron deficiency in patients with BTT may leads to lack of hematopoietic nutrients as well as imbalance in globin chain synthesis, resulting in further reduction in hemoglobin production.

Similar findings were reported by Hinchliffe and Lilleyman [18], who analyzed the frequency of coincident iron deficiency and BTT, and they reported that, the two can coexist. They also suggested that BTT does not prevent ID.

However, in a study by Mehta and Pandya [19], it was observed that patients with BTT had an advantage of maintained iron balance. They suggested that BTT produces slight ineffective erythropoiesis associated with increased iron absorption. Thus, it is likely to give some degree of protection against deficiency of iron.

In a study by Hoorfar *et al.* [20], who assessed iron status in patients with BTT, they stated that BTT may play a role in improving iron status in females, but in men, it can lead to iron overload. They concluded that, iron level should be examined in patients with BTT especially in men, to avoid iron overload.

Our results are in agreement with Arshad *et al.* [15], who studied the effect of iron deficiency in patients with BTT (n=23) on HbA_2 levels, and the selected patients had lower initial levels of serum ferritin less than 20 ng/ml, and HbA_2 levels were less than 3.5%. They added that lower levels of HbA_2 were observed in association with iron deficiency in their patients with BTT. They suggested that the presence of iron deficiency in BTT may mask the diagnosis of BTT.

Consistent with our results, a previous study by El-Agouza *et al.* [7] analyzed the effect of IDA on the levels of Hb, MCV, MCH, serum ferritin, HbA_2, and HbF in patients with BTT with IDA (n=4), where patients were treated with oral ferrous sulfate (325 mg/day) for a period of 20 weeks. They reported a significant increased levels of Hb (10.96±1.12 before and 13.20±0.65 after treatment, $P<0.001$), MCV (72.38±5.15 before and 81.30±5.22 after treatment, $P<0.001$), MCH (23.35±2.23 before and 25.52±1.70

after treatment, $P<0.001$), serum ferritin (3.45±1.73 before and 28.77±14.13 after treatment, $P<0.001$), and HbA_2 (1.89±0.45 before and 2.19±0.53 after treatment, $P<0.001$). However, HbF levels were not significantly different after iron therapy (0.94±0.18 before and 0.95±0.17 after treatment, $P>0.05$). They concluded that iron deficiency may interfere with diagnostic tests of HbA_2, and it must be identified and treated before any diagnostic or therapeutic procedure.Our results are in agreement with a study by Usman *et al.* [21], which included patients with combined BTT and IDA ($n=8$). Patients with the copathological conditions of BTT and IDA were treated with oral ferrous sulfate (325 mg/day) for a period of 20 weeks. They reported that patients with combined BTT and IDA responded to treatment with significant increase in HbA_2 levels at the end of oral iron therapy.

Consistent with our results, a previous study by Keramati and Maybodi [8] evaluated the effect of iron deficiency on HbA_2 levels in patients with BTT and those with coincident BTT and IDA. They found significant differences in HbA_2 values between patients with BTT (5.6±0.9%) and patients with coincident BTT and IDA (4.7±1%). They concluded that IDA can decrease HbA_2 level, which may interfere with diagnosis especially in cases with coincident IDA and BTT. Thus, when BTT with IDA is suspected, it is better to do hemoglobin electrophoresis after treatment of iron deficiency.

However, Passarello *et al.* [16] reported that there could be some problems in the presence of silent beta-thalassemia carrier with ID, as HbA_2 shows normal levels. They stated that lower levels of hemoglobin and MCV, and persistent low levels of MCV and MCH after iron therapy, required further molecular analysis of α and β genes, especially if the partner is a carrier of BTT.

Conclusion

Our results showed that patients with BTT who developed IDA can successfully treated with oral iron therapy for a better quality of life. Oral iron has a good role in improving anemia by significantly increasing hemoglobin levels, red blood cell indices, serum iron, serum ferritin, and HbA_2 levels.

Limitations

The study has some limitations such as the small number of patients with BTT who had IDA.

Recommendations

Further trials with larger number of patients and longer follow-up are recommended.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Dolai TK, Nataraj K, Sinha N, Mishra S, Bhattacharya M, Ghosh MK. Prevalance of iron deficiency in thalassemia minor: a study from tertiary hospital. Indian J Hematol Blood Transf 2012; 28:7–9.

2 Karim MUA, Moinuddin M, Babar SU. Cap+ 1 mutation; an unsuspected cause of beta thalassaemia transmission in Pakistan. Turk J Haematol 2009; 26:167.

3 Bencaiova G, Burkhardt T, Krafft A, Zimmermann R. Screening for β-thalassaemia trait in anaemic pregnant women. Gynecol Obstetr Investig 2006; 62:20–27.

4 Amani MA, Neda G, Sumayh A, Francis JB. Hemoglobin A 2 (HbA 2) has a measure of unreliability in diagnosing β-thalassemia trait (β-TT). Curr Med Res Opin 2018; 34:1–16.

5 Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A(2) value in screening for hemoglobinopathies. Clin Biochem 2009; 42:1786–1796.

6 Harthoorn-Lasthuizen E, Lindemans J, Langenhuijsen MC. Influence of iron deficiency anaemia on haemoglobin A2 levels: possible consequences for ß thalassaemia screening. Scand J Clin Lab Investig 1999; 59:65–70.

7 El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Int J Lab Hematol 2002; 24:285–289.

8 Keramati MR, Maybodi NT. The effect of iron deficiency anemia (IDA) on the HbA2 level and comparison of hematologic values between IDA and thalassemia minor. Int J Hematol Oncol 2007; 27:151–156.

9 Madan N, Sikka M, Sharma S, Rusia U. Phenotypic expression of hemoglobin A2 in beta-thalassemia trait with iron deficiency. Ann Hematol 1998; 77:93–96.

10 Verma S, Gupta R, Kudesia M, Mathur A, Krishan G, Singh S. Coexisting iron deficiency anemia and Beta thalassemia trait: effect of iron therapy on red cell parameters and hemoglobin subtypes. ISRN Hematol 2014; 2014:293216.

11 Abdul Hafeez K, Watshara S, Virapong P, Pornlada N. New bioinformatics-based discrimination formulas for differentiation of thalassemia traits from iron deficiency anemia. Lab Med 2017; 48:230–237.

12 Qureshi TZ, Anwar M, Ahmed S, Khan DA, Saleem M. Serum ferritin levels in carriers of β-thalassaemia trait. Acta Haematol 1995; 94:7–9.

13 Niazi M, Tahir M, e Raziq F, Hameed A. Usefulness of red cell indices in differentiating microcytic hypochromic anemias. GJMS 2010; 8:125–129.

14 Jahan T, Ishaq M, Siddiq A. Anemia in pregnant women; prevalence in Ibn-E-Siena Hospital Multan. Prof Med J 2017; 24:5.

15 Arshad M, Ahmed S, Ali N. Effect of iron deficiency on the phenotype of β thalassaemia trait. J Coll Physicians Surg Pak 2016; 26:230–231.

16 Passarello C, Giambona A, Cannata M, Vinciguerra M, Renda D, Maggio A. Iron deficiency does not compromise the diagnosis of high HbA2 β thalassemia trait. Haematologica 2012; 97:472–473.

17 Saraya A, Kumar R, Kailash S, Sehgal A. Iron deficiency in beta-heterozygous thalassaemia. Indian J Med Res 1984; 79:68–75.

18 Hinchliffe R, Lilleyman J. Frequency of coincident iron deficiency and beta-thalassaemia trait in British Asian children. J Clin Pathol 1995; 48:594–595.

19 Mehta B, Pandya B. Iron status of beta thalassemia carriers. Am J Hematol 1987; 24:137–141.

20 Hoorfar H, Sadrarhami S, Keshteli AH, Ardestani SK, Ataei M, Moafi A. Evaluation of iron status by serum ferritin level in Iranian carriers of beta thalassemia minor. Int J Vit Nutr Res 2008; 78:204–207.

21 Usman M, Moinuddin M, Ahmed SA. Role of iron deficiency anemia in the propagation of beta thalssemia gene. Korean J Hematol 2011; 46:41–44.