RESEARCH ARTICLE

Co-existing iron deficiency/overload in beta-thalassemia trait

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Abstract

Objective: To identify the co-existence of iron deficiency and iron overload in individuals with beta thalassaemia trait.

Methods: The cross-sectional study was conducted at Rehman Medical Institute and Khyber Medical University, Peshawar, Pakistan, September 1, 2015, to December 31, 2017, and comprised individuals with hypochromic microcytic blood picture. Haemoglobin electrophoresis was performed on their blood samples. Serum ferritin levels of subjects with Haemoglobin Subunit Alpha 2 levels between 3.5% and 7% were checked. Data were analysed using analysed using GraphPad Prism v6.

Results: Of the 292 subjects, 159(54.5%) were males and 133(45.5%) were females. Of these, 240 (82.2%) were anaemic and 52 (17.8%) had haemoglobin within the normal range. Serum ferritin level of 55(18.8%) subjects was low and 207(70.9%) were iron-replete. Notably, 30(10.3%) subjects had serum ferritin levels higher than the reference range, and this was more common among adults ($p<0.001$).

Conclusion: Ferritin levels in beta thalassaemia trait can be low, normal or higher than the normal values.

Keywords: Beta thalassaemia trait, BTT, Iron deficiency, Iron overload.(JPMA 69: 806; 2019)

Introduction

Beta-thalassemia syndrome (BTS) is a group of hereditary blood disorders characterised by genetic mutations in β-globin chain genes resulting in reduced or absent β-globin chain synthesis. The imbalanced synthesis of haemoglobin (Hb) beta chains leads to variable phenotypes ranging from severe anaemia to clinically asymptomatic individuals. Approximately 1.5% of the global population are heterozygous for β-thalassemia called β-thalassemia Trait (BTT) with the highest carrier frequency reported in Cyprus, Sardinia and Southeast Asia (14-8%)[1]. In Pakistan and India, the prevalence for carrier rate is estimated to be 5–7% and 2.7–4% respectively, which translates to approximately 5–12 million and 30–48 million BT carriers respectively[1, 2].

Iron overload remains the cause of morbidity and mortality in patients with BT major and thalassaemia intermedia. Repeated transfusions lead to excessive iron stores in the body. Moreover, ineffective erythropoiesis also plays a role in iron accumulation from suppression of iron regulatory protein Hepcidin by candidate modifier factors secreted by hyperplastic erythroid cells in the marrow. Not much is known about the status of iron in carriers of BT mutation. Despite having a mutated β-globin chain gene, these individuals are often clinically asymptomatic. The characteristic haematological features are microcytosis, hypochromia, increased Haemoglobin Subunit Alpha 2 (HbA2) level on Hb electrophoresis[3,4]. Hyperplastic erythroid cells in the bone marrow reflect ineffective erythropoiesis. It can be hypothesised that the ineffective erythropoiesis can render BTT individuals susceptible to iron accumulation.

In Pakistan, nearly 1/3rd of the paediatric population and 1/5th of adult females have iron deficiency[5] and the policymakers have suggested, on the basis of studies carried out,[6,7] global flour fortification with iron. This strategy carries the potential to eliminate iron deficiency in resource-deprived / maldistributed countries. However, it may carry a risk of uncontrolled iron administration to iron-replete individuals. This may have implications for a proportion of nearly 7% BTT population. On one hand, iron-deficient patients will benefit from it, while on the other hand without prior investigation whether the BTT individuals are prone to developing iron overload or deficiency, this may be potentially harmful to such individuals. There are no clear statistics for Pakistan's population about iron status in BTT. The current study was planned to identify the co-existence of iron deficiency and iron overload in BTT individuals.

Materials and Methods

The cross-sectional study was conducted at the Department of Haematology, Rehman Medical Institute, Peshawar, Pakistan, from September 1, 2015, to

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December 31, 2017, and comprised paediatric BTT patients who attended outpatient department (OPD), and parents of children with Beta Thalassaemia Major (BTM). Detailed clinical history of each subject was taken. Those with acute infections or on oral iron in the preceding one month were excluded. Also excluded were those suspected of haemoglobinopathies like compound heterozygous, high foetal haemoglobin (HbF) levels, those with abnormal C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values, or raised white blood cell (WBC) count.

A 2ml sample of venous blood was collected from all those enrolled in the study in purple-top K2 ethylenediaminetetraacetic acid (EDTA) vacutainer tube (BD, USA) for complete haemogram / Hb studies, and 2ml in non-additive tube for serum ferritin assay. Complete haemogram was done using automated haematology analyser Sysmex XN-100, (Sysmex, Japan). Blood smears were prepared and stained with Giemsa stain. Hb electrophoresis was performed by capillary electrophoresis using Sebia capillarys-2 (Evry France). Serum ferritin analysis was performed by chemilluminescent microparticale immunoassay-based technology using Architect Ci-8200 analyser (Abbott laboratories, USA).

Participants with microcytosis (Mean corpuscular volume [MCV]<80 fl) and Haemoglobin Subunit Alpha 2)HbA2) levels of 3.4–7.5 % were labelled as BTT; age and gender-specific serum ferritin levels were used for categorisation of participants to iron-deficient, normal or high-ferritin groups (Males: 17-230ng/ml; Females: 14-150ng/ml).

Data were anonamysed and entered in Excel spread sheet and analysed using GraphPad Prism v6. Analysis of variance (ANOVA) and Spearman correlation were used for comparison of means of more than two groups and correlation of HbA2 levels with Hb and Serum ferritin respectively.

Results

Of the 292 subjects, 159(54.5%) were males and 133(45.5%) were females. The overall mean age was 13.7±14.6 years; 149(39%) aged <5 years, 73(25%) aged 5-16 years, and 105(36 %) >16 years. Mean Hb level was 10.2±1.3g/dl. Overall, 240(82.2%) subjects were anaemic and 52(17.8%) had Hb level within normal range. None of the participants had hepatosplenomegaly or jaundice (Table 1).

Table-1: Age and haematological parameters.

Parameter	Mean ± SD
Age (years)	13.7 ±14.2
Hb (gl/dl)	10.2 ± 1.3
RBC counts	5.7 ± 0.7
MCV	58.5 ± 6.5
MCH	18.1 ± 2.6
MCHC	31.0 ± 2.3
RDW (CV)	17.1 ± 4.5
Serum Ferritin (ng/ml)	73.9 ± 75.9
HbA2 %	5.5 ± 0.87
Haematocrit %	31.9 ± 0.4

Hb: Haemoglobin
RBC: Red blood cells
MCV: Mean corpuscular volume
MCH: Mean corpuscular haemoglobin
MCHC: Mean corpuscular haemoglobin concentration.
RDW: Red blood cell distribution width
HbA2: Haemoglobin Subunit Alpha 2

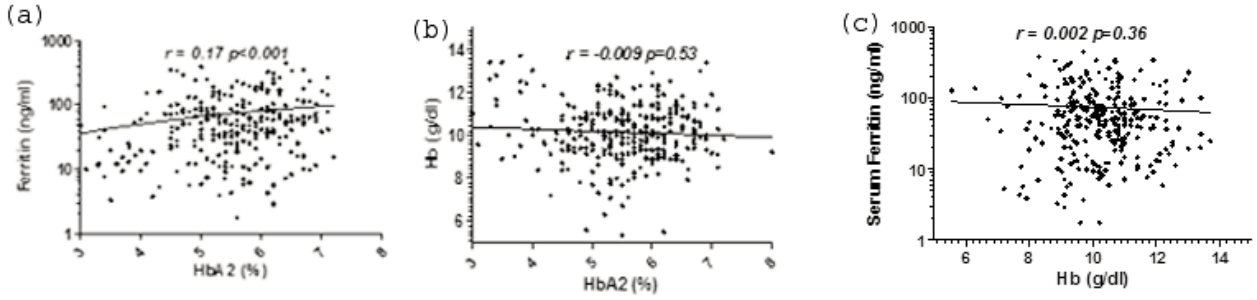

Figure-: (a) serum ferritin levels plotted' against Haemoglobin Subunit Alpha 2 (HbA2) level and analysed using Pearson correlation statistics.
(b) Haemoglobin (Hb) levels of participants plotted against HbA2 levels and analysed using Pearson correlation statistics
(c) Serum ferritin levels plotted against Hb levels and analysed using Spearman's correlation. r=Spearman r

Table-2: Haematological parameters in serum ferritin low, normal and high groups.

Iron status based on serum ferritin levels	Low (n= 55)	Normal (n=207)	High (n=30)	1-way ANOVA
		(mean ± SD)		(p-value)
Age (years)	12.1 ± 11.4	11.4 ± 11.6	33.2 ± 20.3	<0.001
Haemoglobin (g/dL)	10.0 ± 1.2	10.2 ± 1.3	9.6 ± 1.0	0.169
RBC count (109/l)	5.6 ± 0.8	5.7 ± 0.7	5.4 ± 0.7	0.086
MCV (fL)	58.2 ± 8.4	58.5 ± 6.5	59.0 ± 5.8	0.88
MCH (pg)	18.3 ± 3.9	18.0 ± 2.2	18.5 ± 1.9	0.496
MCHC (g/dL)	30.9 ± 2.0	30.9 ± 2.3	31.7 ± 3.9	0.317
RDW-SD (%)	36.6 ± 3.6	35.4 ± 4.3	34.3 ± 1.7	0.231
RDW-CV (%)	18.2 ± 4.2	16.8 ± 4.6	17.5 ± 4.6	0.122
HbA2 (%)	5.2 ± 1.0	5.6 ± 0.8	5.7 ± 0.8	0.007
Serum Ferritin (ng/ml)	9.2 ± 3.7	65.9 ± 41.5	247.1 ± 79.4	<0.001

SD: Standard deviation
ANOVA: Analysis of variation
RBC: Red blood cells
MCV: Mean corpuscular volume
MCH: Mean corpuscular haemoglobin
MCHC: Mean corpuscular haemoglobin concentration.
RDW: Red blood cell distribution width
RDW-CV: Red blood cell distribution width coefficient variation
HbA2: Haemoglobin Subunit Alpha 2

Of the total, 207(70.9%) had serum ferritin levels within normal range, 55(18.8%) had iron deficiency, and 30(10.3%) had the levels high for their age and gender. There was statistically significant difference in age and HbA2 levels among the three groups, while no significant difference was found in Hb level, red blood cell RBC count, MCV, mean corpuscular Hb (MCH) and Red blood cell distribution width (RDW) in the serum ferritin low, normal and high groups (Table 2).

Increasing HbA2 levels were noted with increasing serum ferritin levels ($p<0.001$), but no correlation was found between HbA2 and Hb levels (Figure).

Discussion

Status of iron balance in BTT has been of significant research interest for more than four decades. First investigated in 1978, it was reported that serum ferritin levels in BTT individuals can be low, normal or high compared to the reference range. Interestingly, both iron deficiency and overload were seen in females only. The cause of iron overload was implied to be oral or injectable iron therapy in women[8]. A small family screening showed all BTT individuals of a family had increased liver iron, while normal (not-BTT) members of the family had normal iron stores[9]. Another study showed increased serum and red cell ferritin in 44/101 (43.5%) adults whereas iron deficiency (ID) was only seen in 5/101 (5%) participants [10]. It was reported that ID was common in women of childbearing age[11]. Conversely, a small study showed that BTT males had a higher prevalence of ID compared to control males[12]. Another study demonstrated that 126/463 (27%) BTT individuals had iron deficiency whereas 12/463 (2.6%) had elevated levels of serum ferritin[13] A study on British Asian population found that BTT mutation coexisted with mutations in genes responsible for haemochromatosis (HFE) (HJV, HAMP, SLC40A1, HLA-A3)[14, 15]. However, there is also opposing evidence that the presence of HFE mutation does not cause iron overload in BTT [16].

In our study, 55(19%) individuals had ID whereas 30 (10%) had higher than normal levels of serum ferritin. Most participants with increased levels were adults. This is in keeping with published literature. Homozygous beta-thalassaemia patients develop iron overload. Repeated blood transfusions can be behind this overload. However, other mechanisms may exist. Increased absorption of iron in patients with thalassaemia intermedia has been reported[17]. The spectrum of ID, iron sufficiency and iron overload in BTT implies that there might not be a singular mechanism of iron balance. However, at least a small proportion of individuals may develop iron overload. The most likely explanation is the use of oral iron supplementation in these individuals. Most studies discussed above were hospital-based and may be biased for individuals seeking medical attention for anaemia. Another possible mechanism may include mutations in

hereditary hemochromatosis (HFE) genes[18] or co-existing α-thalassaemia mutations. These have not been tested in relation to iron balance in BTT individuals. The homozygous mutations in HFE gene have a low penetrance and acquired genetic or environmental factors, such as iron therapy, are required to cause iron overload[19]. In a recently published report on British Pakistani population, 8% participants were found to have HFE mutations[20].

Identifying ID in BTT cases is important as it can result in significant reduction in Hb levels without the physician suspecting this treatable factor. BTT individuals with coexisting ID present with a higher degree of anaemia[21-25]. In fact, it has been recommended that a BTT individual presenting with moderate anaemia should be investigated for ID[13]. In our study, Hb levels between participants with low and normal ferritin levels did not differ significantly. Nor was there a correlation between serum ferritin and Hb levels. This lack of correlation between serum ferritin and Hb levels, and Hb and HbA2 levels require further investigation. Likely explanations of this may be the use of iron supplementation or coexisting anaemia of chronic disorders. The enrolled participants represent individuals presenting to the hospital OPDs and may have received supplementation or have comorbidities, but could not be excluded due to recall bias. Another plausible reason might simply be the smaller sample size of this study. A large population-based study will answer this question. Although, perplexing, we still believe it should be reported.

It has been reported that coexisting iron deficiency in BTT may hinder the diagnosis of BTT by reducing the synthesis of HbA2[23]. In our study, serum ferritin levels showed significant positive correlation with HbA2 levels in BTT individuals and significantly lower HbA2 levels were seen in individuals with lower serum ferritin. This finding supports the hypothesis that the degree of iron deficiency can affect the production of HbA2.

The current study is limited by the lack of a control population. How this percentage of iron deficiency or overload is compared to the general population remains to be investigated. A population based cross-sectional study is needed where prevalence of BTT, iron deficiency, coexisting BTT and iron deficiency, and coexisting BTT and iron overload can be determined. It is recommended that any population-based nutritional supplementation strategy be formulated only after the assessment of nutritional status of the population at risk.

Conclusion

A significant proportion of BTT individuals had iron deficiency. A smaller percentage also showed raised serum ferritin. Any population-based intervention should consider this small group of individuals who may develop iron overload with iron fortification of flour.

Disclaimer: None to declare.

Conflict of Interest: None to declare.

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