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Red Cell Parameters in Beta Thalassemia Trait; Comparison between Iron Deficient and Non-Deficient Carriers

Nazish Tahir, Saeed Akhtar Khan Khattak, Ghulam Murtaza Shaikh, Zunera Sajjad, Tamoor Bin Hanif, Nighat Jamal

ABSTRACT

Objective: To compare hematological parameters in iron-deficient and non-deficient carriers of beta thalassemia trait.

Study Design And Setting: Comparative cross-sectional study. This study was conducted at the Department of Hematology at PNS SHIFA Hospital, Karachi for six months (February 2023 to July 2023).

Methodology: This comparative cross-sectional study included a total of 304 cases with beta thalassemia trait and was divided into two groups; the Iron-deficient (ID+) and iron-deficient (ID-) group. The study focused on red cell parameters i.e, Hemoglobin (Hb), TRBC, MCV, MCH and RDW. Haemoglobin Electrophoresis results of both groups were also compared. Descriptive statistics were expressed as mean ± SD and the Chi-square test was assigned. A p-value = 0.05 was considered statistically significant.

Results: Out of the total 304 subjects, 76 (25.0%) had iron deficiency, and 228 (75.0) had sufficient iron stores. Mean age of patients was 18 (Range: 3 – 35) years. HbA levels were similar in both the groups with p-value > 0.05. While all other parameters compared showed marked differences among the two groups and were found to be statistically significant. Hb, TRBC, MCV MCH showed lower values in the ID+ group while RDW was lower in the ID- group.

Conclusion: Red Cell parameters and HBA2 levels in beta thalassemia trait vary significantly among the iron deficient vs non-deficient group. Hence due consideration is needed in screening of beta thalassemia trait in patients with iron deficiency anemia.

Keywords: Beta Thalassemia, Trait, HbA2, Red Cell parameters, Iron deficient carriers, Iron Sufficient carriers.

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INTRODUCTION:

Beta thalassemia trait (BTT) is the most common hemoglobin disorder worldwide with the highest number of cases prevalent in Mediterranean, Middle Eastern, and Southeast Asian regions.[1] The prevalence of BTT worldwide is about 1.5% while in Pakistan the prevalence is as high as 5-8%.[2]

BTT is characterized by the reduced or absent synthesis of beta globin chains of hemoglobin molecules (in a heterozygous state). Electrophoresis of haemoglobin is required for a definitive diagnosis of â-thalassemia trait patients. Hb A2 is normally less than 3.2%, however it is greater than 3.5% in â-thalassemia. HbA2 levels can be detected using different techniques such as High-performance liquid chromatography (HPLC), Cellulose acetate membrane electrophoresis, or Capillary electrophoresis**.** However, in regions where sophisticated diagnostic equipment is unavailable, a simple morphologic criteria has been proposed. It is based on peripheral blood films with microcytic red cells, target cells, and basophilic stippling. The most common technique used for screening is HPLC.[3]

The most prevalent cause of anemia globally is iron deficiency, being particularly common among children and women of childbearing age owing to nutritional deficiencies and menstrual losses respectively. It is also common in old

age due to gastrointestinal blood loss. Body Iron status is determined commonly by serum iron studies that include Serum Ferritin, Iron levels, Total Iron Binding Capacity (TIBC), and Transferrin saturation (TSAT).[4,5] Two of the most common causes of hypochromic, microcytic anemias include beta thalassemia trait and iron deficiency anemia.[6]

Distinguishing between IDA and â-thalassemia trait (â-TT) depends on tests that consume time and money. Not all areas have specialized labs where patients with microcytic anemia can undergo DNA analysis, serum ferritin determination, or hemoglobin electrophoresis. For this reason, different discrimination indices based on various basic red blood cells (RBC) parameters like mean cell volume (MCV), RBC count, and RBC distribution width (RDW) have been suggested since 1973 to differentiate between IDA and â-TT. Red cell parameters in both these conditions (Iron deficiency and BTT) present with specific patterns in the blood complete picture. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are low in both conditions. Comparatively red cell distribution width (RDW) is high in iron deficiency anemia (IDA), while in BTT, RBC count is on the higher side.[7]

It is not uncommon for these two conditions to prevail in the same patient i-e a single patient can be having both BTT and IDA and in that scenario spectrum of red cell parameters could significantly change. In addition, the co-existence of IDA with BTT can mask the HbA2 levels on HPLC.[8] This can lead to a missed diagnosis which can later have detrimental implications as a Beta Thalassemia carrier may get married to another BTT, resulting in the possibility (25% chances) of Thalassemia major in the offspring.[9] Our health care system is not capable enough to adequately manage the increasing number of children with Thalassemia major. A better approach is to identify all the cases of BTT so that necessary actions (genetic counseling and prenatal diagnosis) could be timely done to avoid the far-off obvious implications. It is thus important to differentiate the two conditions and to rule out the possibility of their coexistence by assessing red cell parameters on blood complete picture, an early screening test. The distinction is also critical because Hb will not improve in BTT if it is misdiagnosed as IDA and excessive iron is administered by the attending physician.

Considering above, we would try to assess changes in red cell parameters and HbA2 levels by their comparison in known BTT cases with iron deficient and non-deficient states.[10]

METHODOLOGY:

This comparative random cross-sectional study was conducted at the PNS SHIFA hospital, Karachi for 06 months (February 2023 to July 2023) after getting approval and clearance from the Institutional Ethical Review Committee (ERC/2023/HAEM/37). Sample size (minimum: 114) was calculated through the WHO calculator, keeping the margin of error at 5%, a confidence interval level at 95%, and a BTT prevalence at 5 to 8%.[6] We included 304 individuals with BTT in our study, sampling was done using the nonprobability convenient sampling technique. Known cases of BTT with HbA2 levels greater than 3.5% with and without Iron deficiency as assessed through Serum Iron, TIBC, and Ferritin levels were included in the study. Whereas cases with co-morbidities like infection, chronic disorders and malnutrition were excluded from the study. Data was collected from patients after taking documented consent.

CBC was done on an Automated CBC analyzer (Sysmex Kx-21) within 2-3 hours after collection of the sample in EDTA bottles, to evaluate the hematological parameters (Hb, MCV, MCH, RDW, and TRBC), followed by HbA2, HbA and HbF levels on HPLC and iron studies (Serum Iron, Ferritin levels, TIBC and Transferrin saturation). The Mentzer index of the two groups was also calculated and compared.

The study was conducted in two groups based on Iron profile results, Cases with low serum ferritin levels (<15ng/ml) and transferrin saturation (TSAT= Iron/TIBC x 100) < 20% were taken in the Iron deficient group (ID+). All others were grouped as subjects with sufficient iron levels (ID-).

To determine the effect of iron deficiency on red cell parameters (Hb, MCV, MCH, RDW, TRBC) the ID- group was compared with the ID+ group. The data was analysed using the Statistical Package for Social Sciences (SPSS) version 26:00. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to ensure that the data was normal. The Mann-Whitney U test was employed to detect the difference between the groups since the data observed varied from the normal distribution. A p-value of 0.05 or less was considered significant.

RESULTS:

A total of 304 patients were included in this study, Patients were divided into two groups. Group 1 was iron-deficient (ID+) and Group 2 was iron-sufficient (ID-). Out of the total, 76 (25.0%) patients had iron deficiency, and 228(75.0%) patients had sufficient iron. The median age of the patients was 18, ranging from 3 to 35 years. The distribution of individuals with and without iron deficiency based on gender and age is shown in Table-I. The iron-deficient group had a different gender distribution compared to the iron-sufficient group, and there were differences in the median ages of these two groups as seen in Table-I. The mean of various parameters were compared between two groups, iron-deficient (ID+) and iron-sufficient (ID-), as seen in Table II. Notably, the levels of HbA were found to be similar in both groups, with a p-value > 0.05. In contrast, all other parameters (Hb, MCV, MCH, TRBC, RDW, Iron, TIBC, TS, Ferritin, HBF, Mentzer Index) exhibited statistically significant differences between the two groups, as indicated by p-values < 0.05.

Table 1: Demographic variables of the Patients (n=304)

Demographic variables	Iron-deficient (ID+) (n=76)	Iron-sufficient (ID-). (n=228)
Gender		
Male	31 (40.8%)	134 (58.8%)
Female	45 (59.2%)	94 (41.2%)
Age in years	Median (IQR)	Median (IQR)
	16.0 (26.0-9.0)	19.0 (28.1- 9.0)

Table 2: Comparisons of mean values of red cell parameters and other hematologic findings between the groups (n=304)

Parameters	Iron-deficient (ID+) (n=76)	Iron-sufficient (ID-) (n=228)	p-value
Hb (g/dl)	9.1	11.2	< 0.001
MCV (fl)	56.5	66.7	0.043
MCH (g/dl)	19.32	19.60	0.013
TRBC (x 10^{12} /L)	5.4	6.0	< 0.001
RDW (%)	17.8	16.0	< 0.001
Iron (micromol/L)	5.9	16.3	< 0.001
TIBC ((micromol/L)	83.92	63.3	< 0.001
TS (%)	18.7	26.2	< 0.001
Ferritin (ng/mL)	7.5	22.7	< 0.001
HBA_O (%)	83.3	84.7	< 0.201
HBA_2 (%)	3.8	4.5	< 0.038
HBF (%)	1.5	1.2	< 0.001
Mentzer Index	14.1	10.9	< 0.001

DISCUSSION:

Beta-thalassemias are a group of hereditary blood diseases marked by anomalies in haemoglobin beta chain production, resulting in a wide variety of phenotypes ranging from severe anemia to clinically asymptomatic individuals. The global yearly incidence of symptomatic persons is estimated to be one in 100,000, with the European Union accounting for one in 10,000. There are three types of thalassemia: thalassemia major, thalassemia intermedia, and thalassemia minor. Individuals with thalassemia major typically appear with severe anaemia within the first two years of life. Carriers of BTT are usually symptom free, stresses such as pregnancy can cause the BTT carriers to develop clinically significant anemia sometimes requiring transfusion. Carriers may have mild anemia (Hb: 9-12g/dl). The degree of hypochromia and microcytosis may be comparatively greater than that seen in iron deficiency. Failure to identify BTT antenatally can result in thalassemia major in the offspring, if the partner also carries BTT gene .[11] Thalassemia major patients require regular blood transfusions with many social and economic implications, it occurs when the mutations are inherited in the homozygous pattern.

Iron deficiency anemia (IDA) is the most severe result of iron deficiency and is still regarded the most frequent dietary shortfall globally. The cause of IDA is multi-faceted, but it typically happens when the body doesn't receive enough iron to meet its needs. People who have IDA may not consume enough iron, experience natural iron losses due to aging or reproduction, have difficulties in absorbing or transporting iron, or suffer from chronic blood loss due to illness. Adults with IDA may encounter various negative effects, such as reduced capacity for work or exercise, impaired immune function, gastrointestinal problems, difficulties regulating body temperature, and impaired cognitive abilities. As in BTT, it presents with Low Hb, MCV, and MCH[6,7] TRBC while contrary to BTT, RDW in the case of IDA is usually increased.[12] Blood CP is the initial screening test for hypochromic microcytic anemias. The co-existence of BTT and IDA is a diagnostic challenge as IDA masks HbA2 levels on HPLC leading to misdiagnosis. In one study conducted by El-Agouza *et al*, it was noted that repeating HPLC after iron replenishment resulted in a significant rise in HbA2 levels (from 3.07 to 3.81%) in patients with co-existing IDA and BTT. It is thus very important to understand the spectrum of hematological parameters on CBC in patients with a combination of IDA and BTT. Iron deficiency, once diagnosed, should be adequately managed and patients having borderline HbA2 levels are often advised to repeat HbA2 levels after iron replenishment. Understandably, a multidisciplinary approach in hospitals is needed to address the issue.[13]

In our study, cases were grouped into the iron-deficient and non-deficient group based on the results of serum ferritin levels and TSAT. Ferritin levels may be falsely raised in some cases, as it is an acute phase reactant. Hence, TSAT must be calculated to rule out iron deficiency. Out of the total 304 cases enrolled in our study, 72 (24.0%) had iron deficiency and 228 (76.0%) had sufficient iron levels when we compared the red cell indices of iron-deficient (ID+) vs iron-sufficient (ID-) groups of BTT carriers it was noted that ID+ group showed lower values of Hb, MCV, MCH, and TRBC as compared to the ID- group. However, the ID+ group showed higher values of RDW as compared to the ID- group.

The combined effect of defects in globin chain synthesis and additional nutrient deficiency, on erythropoiesis, leads to further reduction in Hb levels in cases with BTT as concluded in a previous study conducted by Saraya *et al.*[14] Similar results were reproduced in our study.

The Mentzer index of both groups was also calculated using the formula; MCV/TRBC. A value greater than 13 is considered suggestive of IDA and vice versa for BTT. This has previously been proved in many studies. One study by Gonul Aydogan *et al*, [15] stated the sensitivity and specificity of the Mentzer index as 100% and 69.4% respectively. However, not much data is available on the effect of Mentzer index values in combined BTT and IDA. In our study it was noted that subjects in the ID+ group showed a Mentzer index value greater than 13 (mean=14.1) which was considered

statistically significant. Other formulas like Green and King ($MCV^2 \times RDW$)/(Hb × 100), Srivastava (MCH/RBC), Ehsani (MCV-10 × RBC), Shine and Lal (MCV2 × MCH/100), Mentzer (MCV/RBC), England & Fraser formula: (MCV-RBC-5 × Hb-3.4) Ricerca, and 11T score have also been previously used to discriminate the two conditions but their sensitivity is too low.[16] Beyan et al calculated the sensitivity, specificity, positive and negative predictive values of the above mentioned formulae and it was concluded that none of these formulae is superior to RBC value in distinguishing IDA from BTT.[17] Similar results were reproduced in another study by Demir et al in which youden's index of all these formulae was calculated. Youden index shows the sensitivity and specificity of any technique, it was the highest for RBC and RDW and these two indices were considered to be of prime importance in distinguishing between IDA and BTT.[18]

Moreover, the HPLC findings of the two groups were also compared that showed lower values of HbA2 in the ID+ group. It was also noted that the ID+ group showed higher levels of HbF compared to the ID- group. HbA levels were similar in both groups.

In a study conducted by Deniz Aslan *et al*, erythrocyte parameters of IDA in BTT were compared, it was seen that the group with co-existing IDA and BTT did not show a significantly lower value of MCV and RBC as compared to the group with only BTT. It was postulated that this might be due to the fact that concomitant IDA in thalassemia carriers poses a basal stress in erythropoiesis that leads to increased HbF levels thus causing erythrocytosis that restrains microcytosis.[19]

While in another study by Aysel Vehapoglu *et al*, it was concluded that RBC count is not a reliable parameter to distinguish between IDA and BTT as patients with IDA can also have a high RBC count, especially in the beginning of starting Iron replacement therapy. In our study, however, both MCV and RBC counts were significantly lower in the ID+ group as compared to the ID- group.[20]

The co-existence of IDA and BTT is not uncommon and very little data has been previously published on the spectrum of hematological parameters that are observed when both these conditions prevail, our study focused on establishing the changes in RBC parameters when BTT and IDA co-exist.

CONCLUSION

Coexistence of Iron deficiency and beta thalassemia trait results in significant derangements of red cell parameters and HbA2 levels. Therefore, screening of beta thalassemia trait in patients with iron deficiency anemia requires a meticulous approach.

Authors Contributions:
Nazish Tahir: Acquisition, drafting, analysis and interpretation of data for the study
Saeed Akhtar Khan Khattak: Concept, design, analysis and interpretation of the study data
Ghulam Murtaza Shaikh: Revision of data / statistics for intellectual content production and final approval of the version to be published
Zunera Sajjad: Revision of data and proof reading
Tamoor Bin Hanif: Revision and proof reading
Nighat Jamal: Revision and proof reading

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