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Article

Peripheral Blood Erythrocyte Parameters in B-Thalassemia Minor with Coexistent Iron Deficiency: Comparisons between Iron-Deficient and -Sufficient Carriers

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Abstract: Changes in erythrocyte parameters are well known in both β-thalassemia minor (BTM) and iron deficiency (ID) when either is present alone; however, to our knowledge, there has been no study showing the changes when the two conditions coexist. We herein assessed erythrocyte parameters in BTM with coexistent ID. The BTM cases were divided into two groups based on ferritin levels as ID+ and ID−; the ID+ group was then further divided based on hemoglobin (Hb) levels as iron-deficient carriers with (IDA+) and without (IDA−) anemia. When compared to the ID− group, all parameters were significantly different in the IDA+ group except mean corpuscular volume (MCV) and red blood cells (RBC). All parameters except RBC were significantly different between the IDA+ and IDA− groups. Hb, hematocrit (Hct), MCV, and mean corpuscular hemoglobin (MCH) levels in the IDA− group were found to be lower than in the ID− group. Changes in erythrocyte parameters in iron-deficient carriers are critical in screening for BT, particularly for correct formulation of mathematical algorithms utilized by artificial intelligence programs.

Keywords: β-thalassemia minor; iron deficiency; iron-deficient carriers; iron-sufficient carriers; erythrocyte parameters; thalassemia screening

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1. Introduction

Screening for β-thalassemia minor (BTM) involves complete blood count (CBC) and high-performance liquid chromatography (HPLC) [1,2]. Erythrocyte parameters are assessed on CBC, and hemoglobin (Hb)A2 quantification is done in HPLC. Assessment of erythrocyte parameters can be done either directly (mainly mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], red blood cells [RBC] and red cell distribution width [RDW]) or via formulas (e.g., Shine and Lal formula) and artificial intelligence (AI) programs utilizing mathematical methods based on support vector machine (SVM) algorithms [3,4].

When BTM is associated with iron deficiency (ID), HbA2 quantification is misleading (false low levels) [2]; however, the changes in erythrocyte parameters are not clear. This situation has been mostly evaluated with regard to the effect of ID on HbA2 levels or to the improvement in HbA2 levels after iron therapy [5–7]. Studies have been conducted on adults (especially in childbearing women/pregnant women, in whom ID is frequent) or on mixed age groups consisting mostly of adults. There are no studies specifically evaluating erythrocyte changes due to ID in BTM and performed solely in children. Awareness of the changes in this situation is necessary for screening programs, especially to provide correct formulation of AI algorithms. Even the most sophisticated AI programs fail to differentiate those particular carriers due to the lack of data [3]. The aim of this study is to describe the changes in erythrocyte parameters in these special cases as a contribution to carrier screening for BT.

2. Materials and Methods

We evaluated 259 children with BTM (132 males, 127 females; aged 1–17 years, median: 6 years) who were followed in Gazi University Pediatric Hematology Unit from 1 January 2018 to 31 December 2020.

The cases had no comorbidity, malnutrition, or infection. Data were collected from the patients' medical records after obtaining the necessary consent. CBC (Hb, hematocrit (Hct), MCV, MCH, mean corpuscular hemoglobin concentration (MCHC), RBC, and RDW), serum iron parameters (iron, total iron binding capacity [TIBC], transferrin saturation [TS: iron/TIBC X 100], and ferritin), and HPLC (HbA0, HbA2 and HbF) results were assessed. The study group was divided into two groups as ID+ and ID− according to ferritin value (<12 ng/mL, ages < 5 years; <15 ng/mL, ages ≥ 5 years) [8]. ID+ cases were further divided as iron-deficient carriers with (IDA+) and without (IDA−) anemia according to Hb value (<11 g/dl, ages 6–59 months; <11.5 g/dl, ages 5–11 years; <12 g/dl, ages 12–14 years; <12 g/dl women ≥ 15 years; <13 g/dl men ≥ 15 years) [9].

To determine the effect of ID on erythrocyte parameters, the IDA+ group was compared to the ID− group, since IDA+ is the group representing phase 3 ID with detectable changes in erythrocyte parameters [10]. Comparisons between IDA+ and IDA− groups and between IDA− and ID− groups were also done.

The statistical software package SPSS version 16.0 for Windows was applied for the statistical analysis of the results. Descriptive statistics are presented as median (minimum-maximum). Chi-square test was used to compare categorical variables. Since data were not normally distributed, non-parametric tests were used (Kruskal-Wallis test for comparisons of more than two groups and Mann-Whitney test of two groups). Comparisons with parametric tests revealed similar results with similar significance (data not shown). $p < 0.05$ was considered significant.

3. Results

Among 259 cases, 53 were iron-deficient (ID+) and 206 iron-sufficient (ID−). Out of 53 ID+ cases, 28 were anemic (IDA+) and 25 had no anemia (IDA−) (Table 1). Age and gender distribution were similar among all groups, and iron parameters were different between the ID+ and ID− groups ($p > 0.05$ and $p < 0.05$, respectively).

Table 1. Clinical characteristics of the groups.

	β-Thalassemia Carriers *n* = 259		
	ID+ *n* = 53		ID− *n* = 206
	IDA+ *n* = 28	IDA− *n* = 25	
Age (years)	5.5 (1–17)	6 (2–17)	6 (1–17)
Gender			
F	14	10	103
M	14	15	103

ID+, carriers with iron deficiency; ID−, carriers without iron deficiency; IDA+, iron-deficient carriers with anemia; IDA−, iron-deficient carriers without anemia; F, female; M, male.

Comparison of the IDA+ and ID− cases revealed that all erythrocyte parameters except for RBC and MCV (Hb, Hct, MCH, MCHC, and RDW) were significantly different (RBC 5.6 vs. 5.8 $\times$ 10^{12}/L, respectively, p 0.196 and MCV 59.6 vs. 60.5 fl, respectively, p 0.058) (Table 2).

Table 2. Comparisons of erythrocyte parameters and other hematologic findings between the groups.

	β-Thalassemia Carriers ***n* = 259**		
	IDA+	IDA−	ID−
	n = 28	*n* = 25	*n* = 206
Median (min-max)			
Hb (g/dl)	10.5 (8.5–11.9) [a,b]	12.0 (11.2–13.4) [c]	11.1 (7.9–14.7)
Hct (%)	34.0 (28.4–39.1) [a,b]	38.1 (34.8–43.2) [c]	35.3 (25.2–46.8)
MCV (fl)	59.6 (51.0–68.0) [b]	67.0 (56.2–72.2) [c]	60.5 (53.1–75.7)
MCH (pg)	18.2 (15.2–21.0) [a,b]	21.7 (17.4–23.9) [c]	19.0 (15.6–25.1)
MCHC (g/dl)	30.8 (29.2–32.1) [a,b]	31.7 (29.6–33.4)	31.5 (28.6–34.8)
RBC ($\times 10^{12}$/L)	5.6 (5.1–6.8)	5.7 (5.0–7.0)	5.8 (4.5–7.2)
RDW (%)	18.5 (15.4–28.9) [a,b]	16.0 (14.5–22.9)	16.8 (12.8–30.9)
Iron (μg/dl)	50.2 (14.0–130.0) [a]	53.3 (21.0–115.6) [c]	74.0 (11.0–157.0)
TIBC (μg/dl)	317.2 (238.6–600.0) [a]	353.3 (279.0–467.7) [c]	279.4 (160.4–460.3)
TS (%)	15.5 (2.3–40.0) [a]	16.3 (5.8–34.3) [c]	26.4 (2.7–77.0)
Ferritin (ng/mL)	9.0 (3.0–14.0) [a]	9.4 (3.0–13.0) [c]	30.0 (12.0–92.0)
HbA_0 (%)	82.5 (69.3–87.1)	84.9 (77.4–88.2)	83.3 (62.4–96.0)
HbA_2 (%)	4.8 (1.7–6.1)	2.9 (2.3–6.0) [c]	4.8 (2.2–8.7)
HbF (%)	1.6 (0.2–17.2)	1.3 (0.2–6.9)	1.4 (0.1–10.6)

IDA+, iron-deficient carriers with anemia; IDA−, iron-deficient carriers without anemia; ID−, carriers without iron deficiency. Superscript letters indicate statistically significant values ($p < 0.05$). [a] IDA+ vs. ID−; [b] IDA+ vs. IDA−; [c] IDA− vs. ID−.

When IDA− and ID− cases were compared, Hb, Hct, MCV, and MCH were found to be significantly different ($p < 0.05$ for all), while MCHC, RBC, and RDW were similar ($p > 0.05$ for all) (Table 2).

A comparison of IDA+ and IDA− cases showed that all erythrocyte parameters except RBC were significantly different ($p < 0.05$ for all). RBC was 5.6 vs. 5.7 $\times$ 10^{12}/L, respectively (p 0.392) (Table 2).

4. Discussion

Thalassemia is a major health problem, with individual, social, and economic burdens [1]. Eradication is feasible with screening and disease control. Detection of carriers is possible using erythrocyte parameters on CBC [2]. MCV, MCH, RBC, and RDW are generally used for detection, with low MCV, low MCH, high RBC, and normal RDW denoting carrier status.

ID is the most prevalent cause of anemia worldwide, especially among women and children [8,9]. ID develops in sequential changes over a period of negative iron balance and has measurable effects on erythropoiesis and erythroid cells [10,11]. These stages include the iron depletion phase (phase 1), iron-deficient erythropoiesis (phase 2), and finally IDA (phase 3). Phase 1 is characterized by decreased bone marrow iron stores; Hb and serum iron remain normal, but serum ferritin falls. During phase 2, erythropoiesis is impaired; serum iron and TS decrease. During phase 3, anemia develops. As the state of ID proceeds, microcytosis and then hypochromia develop. Finally, ID affects tissues, resulting in symptoms and signs [10].

When BTM and ID coexist, sophisticated AI programs may even fail in differentiation based on erythrocyte parameters, probably due to the lack of data [3]. Our study is the first to specifically investigate the erythrocyte parameters of these particular carriers.

The prevalence of ID in the study group (21%) was consistent with results of previous studies [5–7,12]. The ID+ group theoretically includes all carriers with every phase of ID. When we divide this group according to anemia, the IDA+ group represents the carriers with phase 3 ID and the IDA− group represents carriers with phases 1 and 2. IDA+ is the group with detectable changes in erythrocyte parameters (Hb and others), and its

comparison with the ID− group shows characteristic erythrocyte changes resulting from ID, which are expected as lower Hb, Hct, MCV, MCH, MCHC, RBC, and higher RDW.

Our results were in accordance with this expectation, except for RBC and MCV. Interestingly, RBC and MCV were not as low as expected. The likely explanation is the augmented basal stress erythropoiesis in BTM resulting from tissue hypoxia due to the coexistent ID [13] and the consequent increase in HbF synthesis taking place in F-cells [14]. The former leads to erythrocytosis and the latter restrains microcytosis arising from ID. In accordance with this, the level of HbF in IDA+ cases was numerically higher as compared to levels in iron-sufficient carriers and even to those in IDA− cases. The lower decline in MCV in our study might be due to this situation. The relatively low levels of HbF might be explained by quantitative trait loci (QTLs) [15]. Interestingly, some parameters were similar between IDA+ and IDA− carriers; RBC in IDA+ cases was not as low as expected, probably due, besides the compensatory increase caused by tissue hypoxia, to the lower decline in RBC, since ID was not that severe in the studied cases. Further, some erythrocyte parameters in IDA− group were found to be significantly different as compared to ID− cases (Hb, Hct, MCV, MCH, all lower), though similarities were expected. This suggests that the response to ID on the basis of BTM differs from that of pure ID. Actually, in our clinical experience, there are other examples of conditions in which laboratory hallmarks are not observed when BTM coexists (supplementary data). In the present study, this situation is presented as early changes in erythrocyte parameters, making the difficulties in discrimination inevitable regardless of the phase of ID in this particular patient group. Similarly, in a remarkable screening using SVM algorithms [3] data obtained from both groups with Hb < 9 g/dl, differences between erythrocyte parameters were not significant, and this was attributed to the presence of combined BTM and IDA in both groups. In our study, however, differences were observed before anemia developed. Considering our observation of changes in every phase of ID, in addition to the high frequency of coexistence, the erythrocyte changes in iron-deficient carriers seem to be important in discrimination studies.

Since thalassemia mutations leading to erythrocyte changes are population-specific, AI tools in different populations might be helpful when considering this situation in the future.

In conclusion, much progress has been made to date in the laboratory distinction between BTM and ID. At this point, the troublesome group is the cases in whom the two conditions coexist. Learning the erythrocyte characteristics of these cases can help to achieve the goal of an almost zero negative predictive value (NPV) for an ideal screening program. Our study aimed to describe those parameters of these particular cases as a contribution to screening programs and revealed that in B-TT coexistent with ID, carriers have hypochromic microcytic anemia with elevated RBC and RDW. Further studies will be helpful.

β-thalassemia minor (BTM) has an inherent "potential" and prevails over the coexistent condition (whether Fanconi anemia [FA], vitamin B12 deficiency, or iron deficiency [ID]). The potential might be "F-cell and HbF". Of note, variations of HbF levels are present, suggesting that despite the advances, gaps remain in our understanding of the genetic regulation of HbF and F-cell production in thalassemia.

Here are the examples of our experiences:

Fanconi anemia is one of the diseases in which expected hematological changes are not observed when BTM coexists.

Case example: A 28-month-old boy with Hb 7.9 g/dl, MCV 71.4 fl, RBC count 3.4×10^2/L, RDW 16.8%, HbF 12.1%, HbA2 5%, heterozygous state for the β-thalassemia mutation of IVS-I-110 [G/A] with normal body iron status, and homozygous for the FA mutation of c.1615del;p.D539TfsX66 in the FANCA gene.

In our own clinical experience, vitamin B12 deficiency is another example of conditions in which laboratory hallmarks are not observed when BTM coexists.

Case example: A 4-year-old girl with Hb 11.6 g/dl, MCV 59.7 fl, RBC count 6.5×10^{12}/L, and RDW 17.3%, seemingly indicating pure BTM; however, further testing revealed vitamin

B12 deficiency (158 pg/mL; N: 258–1435 pg/mL) with normal iron status parameters, and heterozygous state for the β-thalassemia mutation of IVS-I-110 [G/A] with HbF 1%, HbA2 4.9%.

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