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ORIGINAL ARTICLE

Status of Red Blood Cell Indices in Iron Deficiency Anemia and β Thalassaemia Trait: A Comparative Study

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Abstract

Background: *Iron deficiency anemia (IDA) and β thalassemia trait (β -TT) are the two important differential diagnosis of microcytic hypochromic anaemia. It is important to distinguish between the above two conditions to avoid unnecessary iron therapy. Red blood cell (RBCs) indices are simple, easy, and cost effective method to get a primary and valuable information regarding the diagnosis of IDA and β -TT.*

Objectives: *This study was aimed to compare the RBC count, hemoglobin (Hb), hematocrit (Hct%), RBC indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and redcell distribution width index (RDWI) in Iron deficiency anemia and β thalassemia trait.*

Methods: *This cross-sectional comparative study was conducted in the department of clinical pathology, Dhaka Shishu Hospital, Dhaka from July 2019 to March 2020. Total 107 patients with microcytic hypochromic anaemia (64 subjects of IDA and 43 subjects of β -TT were tested for RBC count, Hb%, MCV, MCH, MCHC, RDW, and PCV from venous blood by haematology analyser. Serum ferritin was measured by Enzyme Linked Immunosorbent Assays (ELISA). Statistical analysis was performed by SPSS version 22. Statistical significance was set at p value less than 0.05.*

Results: *RBC count, Hb, and Hct,MCV, MCH and MCHC were significantly lower and RDW and RDWI was significantly higher in IDA group than in β- TT group ($p< 0.001$). Similar result was observed in male and female participants when compared them in separate group.*

Conclusion: *The study showed that RBC count, Hb, Hct, MCV, MCH, and MCHC were significantly lower in IDA group than in β-TT group, whereas RDW and RDWI were significantly higher in IDA group than in β-TT group.*

Keywords: *Iron deficiency anemia, β thalassemia trait, red blood cell indices.*

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Introduction

Anemia may be defined as a reduction in the concentration of Hb which leads to reduced oxygen carriage and delivery to the tissues.[1,2] The global prevalence of anemia in children aged 6-59 months is 43%.[3-5] Anemia is considered a public health problem in developing countries, and it has been estimated that 2 billion people suffer from anemia worldwide. The underlying causes of anemia are many, varied and preventable, such as nutritional deficiencies, infections, and hemoglobin (Hb) disorders.[6] Microcytic hypochromic anaemia is a very common haematological abnormality in clinical practice.[7] Two most common causes of microcytic hypochromic anaemia are iron deficiency anemia (IDA) and β thalassemia trait (β -TT), which are sometimes difficult to differentiate clinically.[8,9] IDA in young children is recognized as a major public health issue and the most prevalent form of micronutrient deficiency worldwide.[3] IDA may occur as a result of an iron-deficient diet, intestinal iron malabsorption, and chronic blood loss due to many factors like hemorrhage or hemoglobinuria because of intravascular hemolysis.[10-12]

Thalassemias are defined as a heterogeneous group of genetic disorders of Hb synthesis due to the reduction of one or more of the globin chains production.[13] Thalassemia is a growing global public health problem as it was expected that about 900,000 births of clinically significant thalassemia disorders would occur in the year 2020.[14] It is estimated that about 1.5% of world population are carriers of the associated genetic mutation.[15,16]

IDA and β-TT should be differentiated to avoid unnecessary iron therapy, because iron treatment is indicated in IDA and contraindicated β-TT.[17] Hb electrophoresis is a reliable, rapid, reproducible and easy method to separate various Hb fractions depending on their charge and these fractions are then quantitated.[18] A definitive differential diagnosis between between IDA and β-TT is based on the result of HbA2 percentage, serum iron, and ferritin concentration. Electronic cell counters have been used to determine red cells indices as the first indicator of anemia. To reduce unnecessary investigation, blood indices are used to detect subjects who have a high probability of anemia. To differentiate these two conditions several rapid and inexpensive discriminating indicators have been proposed in large-scale research since 1973. These indices are obtained from automated cell counters that traditionally give parameters like Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red Blood Cell Distribution Width (RDW), Mean Corpuscular Hemoglobin Concentration (MCHC), and Red Blood Cell Count (RBC).[19-23] The modern hematology laboratory uses the automated blood cells analyzed. It gives rapid, cost-effective and accurate analysis of red cell indices which have an important diagnostic utility. Most of these analyzers measure the RBC count, MCV, and Hb concentration. The other indices such as the hematocrit (Hct), MCH, and MCHC are derived from the primary measurements.[24] The MCV is either directly measured by the instrument, or it is calculated by certain formula. The red cell distribution width (RDW) is calculated as standard deviation (SD) of RBC or as a coefficient of variation.[25] This study will describe and compare the RBC indices of IDA group with those of â- TT group to have detail information about the behavior of the RBC indices in the above two different conditions.

Materials and Methods

This cross sectional comparative study was conducted in the department of clinical pathology, Dhaka Shishu Hospital, Dhaka from July 2019 to March 2020. Total 107 children of both sexes with microcytic hypochromic anaemia (64 children with IDA and 43 children with β-TT) age ranged from 1 year to 18 years were included in this study. Patients already on nutritional supplements, having long standing illness or on medication interfering with micronutrient metabolism (e.g. antiepileptic drugs such as acetazolamide, carbamazepine and clobazam, aspirin and antacids containing magnesium hydroxide) and patients who were diagnosed and was having a family history of haematological disorders other than thalassemia were excluded from the study. A questionnaire was used to obtain the data including age and sex. Two ml peripheral venous blood were collected under sterile conditions in an EDTA tubes and another 2 ml venous blood were collected in the other tubes without anticoagulant for serum separation. Serum was separated within 3 hours of collection. The RBC count, the measurements of Hb, MCV, MCH, MCHC, RDW, and packed cell volume (PCV/Haematocrit) were obtained by haematology analyser: Mythic -22 using reagent Kits (Diluent, Cleaner, Lytic). Serum ferritin was

measured by Enzyme Linked Immunosorbent Assays (ELISA) using reagent kits (IMMULITE and IMMULITE 1000 system). According to the WHO guidelines, anemia is defined as Hb <11g/dl; and iron deficiency as Serum Ferritin <12 μg/l in children <5 years old and <15 μg/l in children older than 5 years in both male and female group.[26] Statistical analysis was performed using statistical package for social science (SPSS) software version 22 (SPSS Inc. Chicago, IL). The RBC count, concentrations of Hb, Hct, MCV, MCH, MCHC, RDW and RDWI were expressed in mean ±SD. Descriptive statistics were applied to describe the value derived from tests. The mean concentration of Hb, Hct, RBC count and red cell indices were compared using independent sample Student *t* test between IDA group and β TT group. Statistical significance was set at *p* value less than 0.05.

Result

Total 107 patients with microcytic hypochromic anaemia were included in the study. Among them 64 subjects were IDA and 43 subjects were β-TT. The age of patients were from 1 year to 18 years, both male and female children were included in the study. The mean ± age of IDA group and β -TT group was 4.31±4.58 (range 1-18 years) and 5.07±4.72 (range 1-17) years. There was no significant difference in age between two groups (p=0.409) (Table I).

There were 36 males and 28 females in IDA group and 22 males and 21 females in β-TT group. There were no significant difference between two group (p=0.605). So, IDA group and β-TT group was age and sex matched (Table II).

The comparison between IDA group and β-TT group in regards to RBC count, Hb concentration, hematocrit and red cell indices is shown in Table III. The mean± RBC count (4.42±0.52 vs 5.35±0.74) x 10^{12}/L, p<0.001, 95% CI, -1.075,-0.506) Hb concentration (7.25±1.30 vs 10.49±1.62 g/dl, p<0.001, 95% CI, -3.705,-2.308) and hematocrit (24.36±2.99 vs 32.62±4.81%, p<0.001, 95% CI, -5.832,-5.760) were significantly higher in β -TT group than IDA group. The mean± MCV (55.31±5.36 vs 61.08±5.38, p<0.001, 95% CI, -3.747,-3.703), MCH (16.47±2.81 vs 19.68±2.26, p<0.001, 95% CI, -1.992,-2.036), MCHC (29.38±2.18 vs 32.27±1.57, p<0.001, 95% CI, -2.289,-2.314) were significantly higher in β -TT group than IDA group (Table III). However, the mean± RDW (20.53±1.93 vs 16.68±1.88, p<0.001, 95% CI, 4.678, 4.683), RDWI (254.95±51.68 vs 198.95±55.39, p<0.001, 95% CI, 82.178, 82.411) were significantly higher in IDA group than β -TT group (Table III).

Table I
Age distribution of IDA group and β-TT group

Group	Total number	Age range (years)	Mean ±SD (years)	p value
IDA	64	1.0-18.0	4.31±4.58	0.409
β-TT	43	1.0-17.0	5.07±4.72	

Table II
Sex distribution of IDA group and β-TT group.

Group	Total number	Gender		p value
		Male	Female	
IDA	64	36	28	0.605
β-TT	43	22	21	

Table III
Comparison of RBC count, Hb, Haematocrit and red cell indices between IDA and β-TT group

Variable	Iron deficiency anaemia (IDA) (n=64) Mean ±SD	β-thalassemia trait (β-TT) (n=43) Mean ±SD	p value	95% Confidence interval
RBC count (x10^{12}/L)	4.42±0.52	5.35±0.74	<0.001	-1.075,-0.506
Hb (g/dl)	7.25±1.30	10.49±1.62	<0.001	-3.705,-2.308
Hematocrit (%)	24.36±2.99	32.62±4.81	<0.001	-5.832,-5.760
MCV (fl)	55.31±5.36	61.08±5.38	<0.001	-3.747,-3.703
MCH(pg)	16.47±2.81	19.68±2.26	<0.001	-1.992,-2.036
MCHC(g/dl)	29.38±2.18	32.27±1.57	<0.001	-2.289,-2.314
RDW%	20.53±1.93	16.68±1.88	<0.001	4.678, 4.683
RDWI	254.95±51.68	198.95±55.39	<0.001	82.178, 82.411

Discussion

The most commonly encountered disorders with mild microcytic anemia are IDA and β-TT. Differentiation of this two condition is very important because their prognosis and treatment are different. The first step to diagnose microcytic anemias is to analyze blood samples and to determine the erythrocyte indexes using cell counters.[26]

In our study we found that RBC count, Hb and Hct values were higher in β-TT group than IDA group (Table III). Our findings were similar to the findings of Vehapoglu et al.[19] They considered RBC count a valuable index and found higher RBC count in 64.1% of 290 children with microcytic anemia at the time of diagnosis. However, the frequency of high RBC count was 29.4% in children with IDA. Which indicates that more number of patients with β-TT have higher RBC counts than patients with IDA. The authors also observed that the RBC count was increased at the initiation of iron therapy in patients with iron deficiency anemia and decreased by the end of therapy.

In our study we found that the Hb concentration was more in β-TT than iron deficiency anemia (10.49±1.62 g/dl vs 7.25±1.30 g/dl, p<0.001).and RDW is greater in IDA than beta thalassemia trait patients (20.53±1.93 vs 16.68±1.88, p<0.001). These findings were consistent with findings of Miri-Moghaddam et al[27], Belisario et al[28] and Urrechaga et al[29]. They observed that erythrocytosis (increased RBC) and mild anemia are characteristics of β-thalassemia trait, and the erythrocytes are usually more microcytic (reduced MCV) than in with iron deficiency anemia; whereas in with iron deficiency anemia, the level of anisocytosis (RDW) is greater, along with lower Hb levels when compared with those of β-thalassemia.[27-29] These higher values are related to the disease pathophysiology as excess globin chain leads to an ineffective erythropoiesis, resulting in increased RBC production trying to compensate for anemia.[30] RDW and RDWI were significantly higher in IDA group than in β-TT group (Table III) and that agrees with other studies. RDW showed a highly significant difference between β-TT and IDA (p <0.001). The highest RDW was found in IDA followed by the β-TT reflecting more anisocytosis in IDA than β-TT. Rahim et al[31] also found that RDW was higher in IDA than in β-TT patients. In β-thalassemia trait, almost all RBC are microcytic because deficient synthesis of globin chains resulting from thalassemia mutations expresses itself in all of the RBC precursors. Consequently, RDW values are relatively constant.[32] IDA is progressive rather than stable and if the patient suffers from chronic blood loss. Furthermore, IDA leads to abnormal erythropoiesis those results in increased variation in shape and size: Poikilocytosis and anisocytosis.[33]

Conclusion

The study showed that RBC count, Hb, Hct, MCV, MCH, and MCHC were significantly lower in IDA group than β-TT group, whereas RDW and RDWI was significantly higher in IDA group than β-TT group.

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