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Original Research Paper

Utility of Red Blood Cell Indices in the Diagnosis of β-Thalassemia Minor

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ABSTRACT:

Background: β-thalassemia minor (β-TM) and iron deficiency anemia (IDA) are the most common causes of hypochromic microcytic anemia. The objective of this study was to determine the hematological features of β-thalassemia minor and to determine the sensitivity and specificity of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell count (RBC), red cell distribution width (RDW), and Mentzer index (MI) as screening tools for β-thalassemia minor. **Methods**: A prospective study was conducted at the Teaching Hospital for Children in Iraq. A total of three hundred patients aged 2- 12 years were included. Patients were divided into two groups: the β-thalassemia minor group and the IDA group. Red blood indices were taken as part of the complete blood count. Calculations were made for sensitivity, specificity, positive predictive value, and negative predictive value. **Results**: In the β-thalassemia minor group, the mean (RBC count and RDW) were $5.96\pm0.71\times10^6$/μL and 15.33±1.29%, respectively (p <0.0001). The mean MCV was 61.97±5.20 fL ($p < 0.01$). The sensitivity of the RBC count was 90%, while the specificity of RDW was 98.57%. Mentzer index in thalassemia minor patients was below 13 in 144 (89%). **Conclusion**: MCV and MCH were found to be highly sensitive screening tests for the detection of β-thalassemia minor, followed by RBC count and MI, while RDW was found to be highly specific for the diagnosis of β-thalassemia minor.

Keywords: β-thalassemia minor, Iron deficiency anemia, Red blood cell indices.

INTRODUCTION:

Globally, thalassemia disorders are the most common hereditary diseases, which represent a heterogeneous group of inherited anemias caused by defects in the production of one or more globin chains. Thalassemias have been reported in nearly every region of the world, especially in the Mediterranean basin and parts of Africa and Asia (1, 2). About 1.5% of the world's people are carriers of the β-thalassemia gene (3). The heterozygous disease, in which only one defective beta gene is acquired from the parent, is known as β-thalassemia minor and is usually discovered by chance through routine tests or surveys and during family studies of individuals with severe types of the disease (4, 5). Usually, individuals with the β-thalassemia trait are asymptomatic and misdiagnosed as having iron deficiency anemia. It's crucial to do investigations to differentiate between these two conditions in order to provide genetic advice to thalassemia trait carriers, prevent excessive tests in people who are mistakenly believed to have iron deficiency, and avoid unnecessary iron therapy (6). β-thalassemia minor could be excluded by hemoglobin electrophoresis, which is costly and unavailable in peripheral regions of countries with a high prevalence of thalassemia. A number of complete blood count indices have been used since the seventies of the last century as an easy and low-cost test to distinguish between β-thalassemia minor and iron deficiency anemia (7, 8). In this study, we evaluate the role of red blood cell indices in the diagnosis of β-thalassemia minor.

METHODS:

A prospective study was conducted at the Teaching Hospital for Children between October 2013 and December 2014. A total of three hundred patients (2-12 years old) were included in the study. Data was collected (with the patients' and their parents' permission) from the thalassemia center and outpatient clinic by using a questionnaire that includes: age, gender, history of [recent illness, drug use, and blood transfusion in the last three months], and the results of: RBC count, hemoglobin (Hb), packed cell volume (PCV), MCV,

MCH, mean corpuscular hemoglobin concentration (MCHC), RDW, serum ferritin, and hemoglobin electrophoresis.

Patients were divided into two groups: the β-thalassemia minor group and the iron deficiency anemia group. The β-thalassemia minor group consists of 160 patients, all of whom were confirmed to have β-thalassemia minor (HbA2 greater than 3.5% and normal serum ferritin). The iron deficiency anemia group consists of 140 patients, all of whom were confirmed to have IDA: Hb A2 less than 3.5%, serum ferritin < 12 ng/ml for patients ≤ 5 years old, and < 15 ng/ml for patients > 5 years old (9).

In this study, the patients at age ≤ 6 years were considered to have hypochromic microcytic anemia when Hb < 10.5 g/dl, MCH < 24 pg, and MCV < 75 fL, and at age > 6 years when Hb < 11 g/dl, MCH < 25 pg, and MCV < 77 fL (10, 11, 12).

Mentzer index (MI) is calculated by using this equation: MCV/RBC count. MI above 13 is consistent with IDA, and below 13 is consistent with β-thalassemia minor (13). The following formulas were used to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV):

Sensitivity = [true positive / (true positive + false negative)] × 100

Specificity = [true negative / (true negative + false positive)] × 100

PPV = [true positive / (true positive + false positive)] × 100

NPV = [true negative / (true negative + false negative)] ×100

Exclusion Criteria:

None of the patients in this study had combined β-thalassemia minor and IDA or other hematological disorders; also, none of them had received iron supplementation or a blood transfusion in the last three months.

Statistical analysis of the data was done using SPSS (version 20). A *p* value < 0.05 is considered to be significant. This study was approved by the local ethical review board of the hospital.

RESULTS:

A total of 300 patients with hypochromic microcytic anemia were enrolled in this study; 147 (49%) were male and 153 (51%) were female. The β-thalassemia minor group consists of 160 (53.3%) patients; 87 (54.4%) were male and 73 (45.6%) were female. The iron deficiency anemia group consists of 140 (46.7%) patients; 60 (42.8%) were male and 80 (57.2%) were female [Figure 1]. Regarding β-thalassemia minor, the mean age of patients was 6.91 ± 2.67 years, while in the IDA group it was 5.88 ±2.71 years [Figure 2].

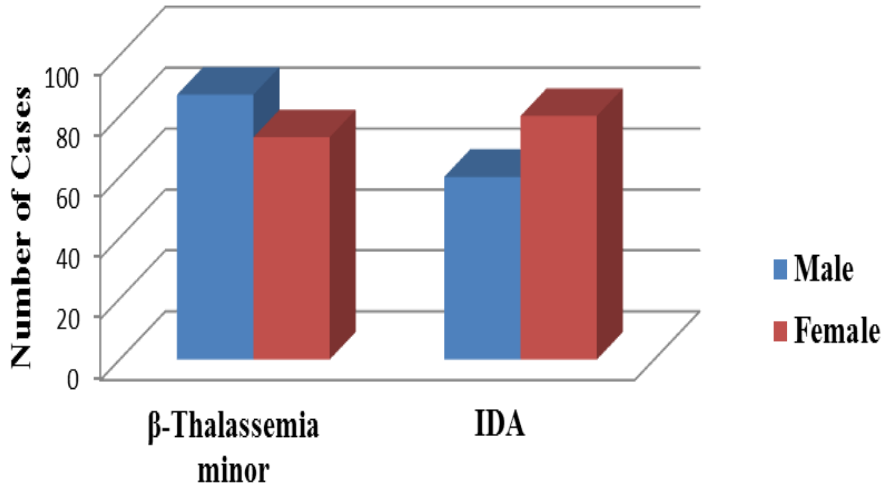

Figure 1. Gender Distribution among the Study Groups

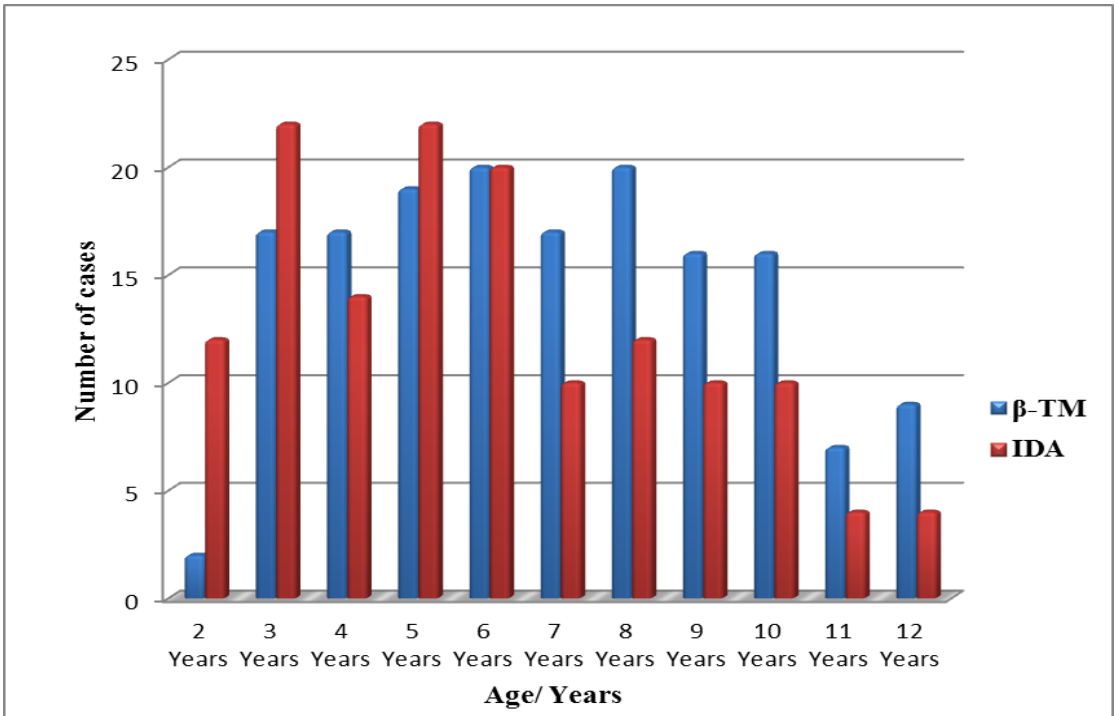

Figure 2. Age Distribution among the Study Groups

Thirty-seven (23.1%) children with β-thalassemia minor were anemic for their age, while 123 (76.9%) were non-anemic. The mean Hb for the β-thalassemia minor group was 11.63±1.33 g/dL, while the minimum and maximum Hb were 8.50 and 14.10 g/dL, respectively. In β-thalassemia minor patients, the mean MCV was 61.97±5.20 fL ($p < 0.01$) and ranged between 46.2 and 74.2 fL, while the mean MCH was 20.10±1.85 pg and ranged between 10.8 and 22.9 pg. None of the patients had MCV or MCH within the normal limit. The RBC count ranged between 4 - 7.90 × 10^6/µL, with 90% of patients having a RBC count greater than 5 × 10^6/ µL ($p < 0.0001$). The mean RDW was 15.33±1.29% ($p < 0.0001$), and in 134 (83.8%) patients, the RDW was more than 14%. The mean HbA2 was 5.51±0.80% ($p < 0.0001$). There is a very highly significant difference in RBC, Hb, and RDW ($p < 0.0001$) between the β-thalassemia minor group and the IDA group, whereas MCV was highly significant ($p < 0.01$). MCH and MCHC had a non-significant difference [Table 1].

Table 1. Hematological Data of the Study Groups

	β-Thalassemia Minor		Iron Deficiency Anemia		
	Mean ±SD	**Range**	**Mean ±SD**	**Range**	***P* value**
Hb (g/dL)	11.63±1.33	8.50 - 14.10	9.49±0.83	6.60 - 10.7	< 0.0001
RBC (10^6/ µL)	5.96 ±0.71	4 - 7.90	4.52±0.55	2.72 - 5.64	< 0.0001
MCV (fL)	61.97±5.20	46.2 - 74.2	63.42 ±5.52	49.7 - 72.9	< 0.01
MCH (pg)	20.10±1.85	10.8 - 22.9	20 ±1.99	15.8 - 23.8	< 0.33
MCHC (g/L)	32.57±2.45	23.4 - 36.7	32.61±2.01	27.10 - 36	< 0.44
RDW (%)	15.33±1.29	11.7 - 17.9	17.39±1.81	14 - 23.40	< 0.0001
HbA2 (%)	5.51±0.80	3.70 - 7.3	2.19±0.42	1.20 - 3	< 0.0001

Mentzer Index in the β-thalassemia minor group was below 13 in 144 (89%) patients [Figure 3]. RDW demonstrated the highest specificity (98.57%) but had very low sensitivity in recognizing β-thalassemia minor. All patients had low MCV and MCH; hence, the sensitivity of MCV and MCH was 100% [Table 2].

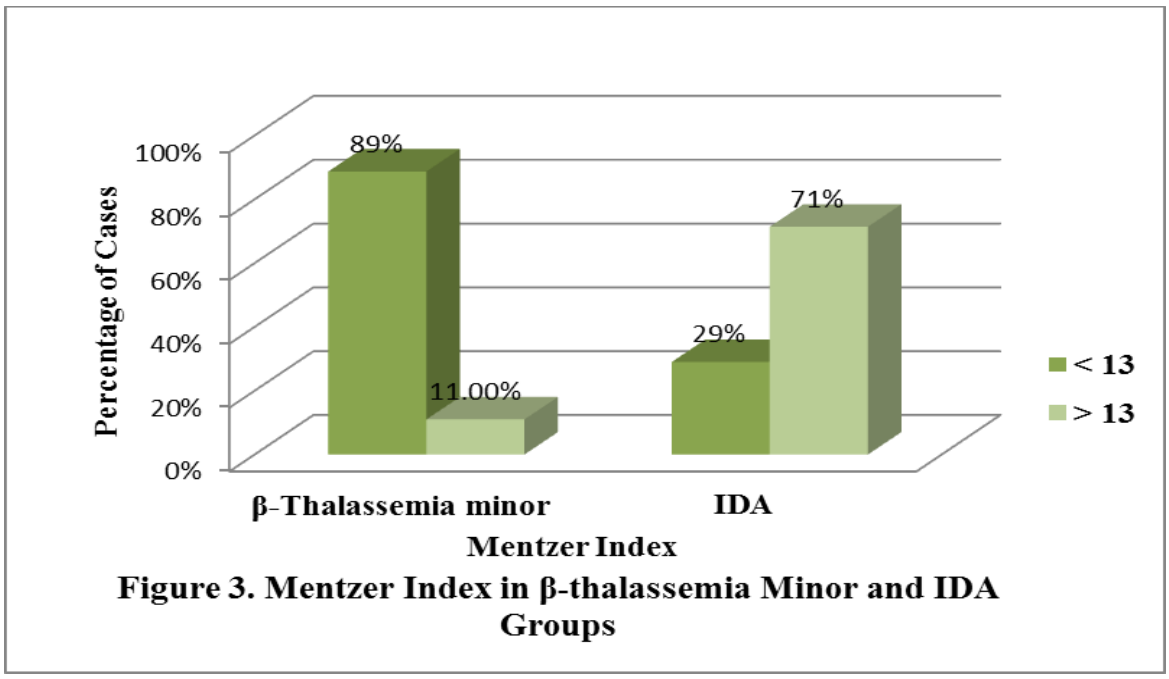

Figure 3. Mentzer Index in β-thalassemia Minor and IDA Groups

Table 2. Sensitivity, Specificity, PPV and NPV for β-thalassemia Minor (β-TM) and IDA Groups

	Cut-off Values	Sensitivity	Specificity	PPV	NPV
RDW %					
β-TM	<14	16.25%	98.57%	96.2%	33.99%
IDA	>14	98.57%	16.25%	33.99%	96.2%
MI					
β-TM	<13	88.75%	71.41%	87.65%	73.5%
IDA	>13	71.41%	88.75%	73.5%	87.65%
RBC(10^6/µL)					
β-TM	> 5	90%	82.85%	92.30%	78.37
IDA	<5	82.85%	90%	78.37%	92.30%

DISCUSSION:

Thalassemia is a prevalent autosomal recessive disease that places huge financial problems on communities and also leads to significant morbidity and mortality (14). A key strategy for preventing β-thalassemia major in succeeding generations is to detect the incidence of β-thalassemia minor (15). For years, RBC indices have been investigated as screening tools for the identification of β-thalassemia minor and might at least alert doctors to the probability of β-thalassemia minor (16). Since the purpose of RBC parameters is to identify persons who have a high possibility of carrying β-thalassemia minor, the ideal parameter should have the highest sensitivity to identify nearly all β-thalassemia cases. Furthermore, specificity needs to be strong enough to prevent measuring HbA2 in a lot of samples that shouldn't need additional testing [false positives] (17).

Iron deficiency anemia and β-thalassemia minor are the

most frequently occurring types of hypochromic microcytic anemia; therefore, distinction between them is crucial to prevent unwarranted iron usage and incorrect diagnosis of β-thalassemia minor (18). In our study, we found that the mean MCV of the β-thalassemia minor group is 61.97±5.20 fL, which is similar to the result of Vehapoglu et al. (60.11 ± 3.49 fL), while Maleki et al. result was slightly higher (64.3±5.5 fL). The mean MCH in our study is 20.10±1.85 pg, which is comparable to Maleki et al. and Vehapoglu et al. results of 19.3±2.55 pg and 18.9±1.37 pg, respectively (7, 15). Maleki et al. mentioned that MCH and MCV were sensitive screening tests for β-thalassemia minor (15). On the other hand, a study by Li et al. found that the sensitivity of MCV and MCH in β-thalassemia minor was 99.3% and 99%, respectively, which is comparable to our result of 100% for both MCV and MCH (19). Although MCV and MCH were appropriate sensitive screening tests for β-thalassemia minor, their specificity was low because β-thalassemia minor and IDA are common causes of hypochromic microcytic anemia (20).

A helpful diagnostic test that is used for β-thalassemia minor is the RBC count, as β-thalassemia minor causes a microcytic anemia with an elevation in RBC count, while IDA is associated with a decline in RBC count that is related to the degree of decrease in concentration (21). Vehapoglu et al. found that the mean RBC count in the β-thalassemia minor was 5.56±0.4×106 /µL, and sensitivity and specificity were 94.8% and 70.50%, respectively (7). While Shen et al. found that the mean RBC count was 5.40±0.58×106/µL, sensitivity and specificity were 82.7% and 83.2%, respectively (22). In our study, the mean RBC count was 5.96±0.715×106/µL, the sensitivity was 90%, and the specificity was 82.85%. Red cell distribution width is a measurement of anisocytosis used to distinguish between β-thalassemia minor and IDA (8). Rathod et al. and Maleki et al. reported that the mean RDW in β-thalassemia minor was 14.47 ± 0.28% and 16.1 ±1.4%, respectively, which is nearly comparable to our results [Table 1] (23, 15). On the other hand, Shen et al. mentioned that the mean RDW was 17.03 ± 1.80%, which is slightly higher than our result (22). The sensitivity and specificity of RDW in our study were 16.25% and 98.57%, respectively, which means that RDW was highly specific for β-thalassemia minor, which is parallel with the result of Shen et al., who found that the sensitivity was 0.0% and the specificity was 99.4% (22). RDW offers some important but limited information about the classification of microcytic anemia because it consistently increases in all cases of microcytic anemia (24).

Mentzer index is used to distinguish between microcytic anemia caused by thalassemia and IDA (25). In our study, the sensitivity of MI in β-thalassemia minor was 88.75% and the specificity was 71.41%. Niazi et al., Shen et al., Rahim et al. and Vehapoglu et al. found that the sensitivity of MI was 98%, 92.1%, 93%, and 98.7%, respectively, while the specificity was 81%, 63%, 85%, and 82.3%, respectively (20, 22, 26, 7) . Therefore, MI is more sensitive than specific for the diagnosis of β-thalassemia minor.

CONCLUSION:

In conclusion, MCV and MCH were found to be highly sensitive screening tests for the detection of β-thalassemia minor, followed by RBC count and MI, while RDW was found to be highly specific for the diagnosis of β-thalassemia minor. RBC indices provide a rapid, low-cost, and reliable test for the detection of β-thalassemia minor; therefore, people with hypochromic microcytic anemia living in countries located in the thalassemia belt should be screened for β-thalassemia minor to prevent thalassemia major in subsequent generations. Premarital screening for β-thalassemia minor should be mandatory in our country, like in other nations with a high prevalence of thalassemia.

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