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Research Article

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Value of Cell Counter-Based Parameters and Formulas in Detection of β-Thalassemia Minor, the experience of a single Haematological Centre in Iraq

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ABSTRACT

Background: Various indices derived from red blood cell (RBC) parameters have been described for distinguishing beta thalassemia minor and other types of hypochromic microcytic anemia. Objective: The study is aimed at investigating the diagnostic reliability of different RBC indices and formulas in differentiation between beta thalassemia minor and other types of hypochromic microcytic anemia. Subjects and Methods: This is a cross-sectional study which was carried out since first of Jan 2011 to end of December 2011 on 171 children with hypochromic microcytic anemia in Kut Oncology Centre, Wasit, Iraq. Results: There was a statistical significant difference between thalassemic group and other groups regarding blood indices as well as the eight formulas which were used. The highest correctly identified patients (PCIP) was reported for RBCs count (84%) with sensitivity and specificity of 96.3%. The Youden's index for RBCs was 58.2 which is the highest value compared with other seven parameters or indices which were used in this study. The second highest Youden's index was for G & K index, with 78.4% PCIP, and sensitivity and specificity of 98.2%. Youden's index of red cell distribution width (RDW) was the lowest value compared to other values used in this study as well as the lowest percentage of correctly identified patients (65%). The sensitivity and specificity of RDW for BTM was 86.1%. Conclusion: According to this study, cell counter-based parameters and formulas, particularly RBCs, and Green and King index are superior to all other methods examined for distinguishing between thalassemia trait and other hypochromic microcytic anemia; while, RDW was inadequate and ineffective for that purpose.

Key words: *Beta thalassemia minor, anemia, RBCs indices.*

INTRODUCTION

Hemoglobinopathies impose a significant problem on global healthcare, and Pathological hemoglobin (Hb) gene was reported in about 5–7% of the worldwide population. It mainly includes the structural Hb variants and different forms of thalassemia [1]. Thalassemia usually has a high predominance in the Mediterranean area, countries in the Middle East, but nowadays population migration has spread thalassemia genes over nearly the entire world [2]. Early and accurate detection of various Hb variants including beta-thalassemia trait (BTT) can avoid the occurrence of serious sicknesses such as thalassemia major in newborns [3]. In the countries with high prevalence of microcytosis and hypochromia and low-income resources, Hb analysis with HPLC and iron study are expensive for public and not available regularly in low-resource settings; accordingly, the automated blood cell counter is widely used in routine practice [4].

Health is on a continuum—one does not arrive at good health accidentally. Personal health begins before birth and continues throughout a person's life [5]. Beta thalassemic patients are at high risk of liver fibrosis because of a high range of hemoglobin in liver, further their livers are more vulnerable than non-thalassemic patients [6]. Early detection thalassemia trait can be a diagnostic dilemma, as many conditions share many characteristics.

Correct diagnosis in patients with microcytic anemia is essential, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in the framework of premarital counselling in high-prevalence areas [7]. Many discrimination indices derived from red blood cell (RBC) indices, such as mean corpuscular volume (MCV), mean corpuscular Hb (MCH), RBC count, and red cell distribution width (RDW) have been advocated as simple and inexpensive tools. To differentiate between BTT and other causes of hypochromic microcytic anemia, particularly for the population in developing countries where the resources are limited [8], the choice of red cell indices, as well as the cut-off values to be used for distinguishing thalassemic from non-thalassemic microcytosis, is controversy. The screening reliability of these indices differs from one country to another. The published cut-off values and interpretations are principally based on the Western population studies [9].

PARTICIPANTS AND METHODS:

During the period from the first of Jan 2011 to end of December of 2011, a cross-sectional study was carried out on 171 patients who referred as cases of hypochromic microcytic anemia to Kut Oncology Centre, Wasit, Iraq.
Investigations were done on 171 patients as complete blood count (CBC) analysis. The hematological parameters, including red cell indices, were measured by an automated blood counter (Sysmex KX-21).
Mean corpuscular volume (MCV) < 80 fL at age > 6 years or MCV < 70fL at age < 6 years was used as definition for LRCI microcytic anemia. The HPLC evaluation was done in a Bio-Rad variant Hb testing system with B-thalassemia short program using variant beta-thalassemia short program pack. Hb A2 level between 4.0% and 9.0% was considered as a case of beta thalassemia minor [10].
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden's index were calculated for eight discrimination indices. Each measure is as follows:

Sensitivity = TP/(TP+FN) × 100
Specificity = TN/(TN+FP) × 100
PPV = TP/(TP+FP) × 100
NPV = TN/(TN+FN) × 100
YI = (Sensitivity + Specificity) - 100

For each discrimination index which was used in the study, the values in the published reports were applied: Mentzer index [11], Green and King index [12], RBC distribution width index [13], Ricerca (R) index [14], Keikhaei index (KI) [15], and Ehsani et al index (EI) [16]. RBC count and RDW were evaluated and compared. Mean and standard deviation (SD) were used to express data. Computerized statistical package for social sciences (SPSS) version 20.0 (SPSS, Chicago, IL, USA) software was used along with independent sample t-test for the two groups of anemic patients. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION:

From first of Jan to end of December of 2011, 171 patients who referred to Hematology center in Wasit, Iraq as cases of hypochromic microcytic anaemia were screened to exclude thalassemia minor. Eight parameters were used with specific cut off point as showed in Table 1.

Table 1: The cut-off point of blood indices in the patients with BT and other LRCI anemia

Indices	IDA	BT
Red blood cell count (RBC) $x10^{12}$/L	< 5	> 5
RBC distribution width (RDW) %	>16	<16
Mentzer index (MI) = MCV/RBC	> 13	< 13
Green and King (G&K) index = MCV^2 × RDW/100Hb	> 65	< 65
RBC distribution width index (RDWI) = MCV × RDW/RBC	> 220	< 220
Ricerca (R) index = RDW/RBC	> 3.3	< 3.3
Keikhaei index (KI) = Hb×RDW×100/ $(RBC)^2$×MCHC	>21	<21
Ehsani et al index (EI) = MCV–10×RBC	>15	<15

Hematological difference between anemic patients with thalassemia minor and other types of low red cell indices is shown in Table 2. Although both groups were hypochromic microcytic anemia patients, there was a statistical difference between two groups regarding blood indices as well as the eight parameters used in this study.

Table 2: Hematological parameters of the patients with BT and other LRCI anemia

Item	diagnosis sorting	N	Mean	Std. Deviation	P value
RBCs	BTM	108	5.8	0.5	0.00
	Other LRCI	63	4.8	0.75	
HCT	BTM	108	37.9	4.8	0.00
	Other LRCI	63	29.6	5.4	
Hb	BTM	108	11.5	1.8	0.00
	Other LRCI	63	8.4	2.2	
MCV	BTM	108	66.0	5.9	0.00
	Other LRCI	63	62.4	7.3	
MCHC	BTM	108	29.9	1.9	0.00
	Other LRCI	63	27.9	3.6	
MCH	BTM	108	19.6	2.5	0.00
	Other LRCI	63	17.7	4.0	
RDW	BTM	108	12.4	1.7	0.00
	Other LRCI	63	14.4	3.6	
MI index	BTM	108	11.4	1.4	0.00
	Other LRCI	63	13.3	3.2	
KI	BTM	108	14.0	1.7	0.00
	Other LRCI	63	19.3	7.9	
RDWI	BTM	108	140.3	17.3	0.00
	Other LRCI	63	194.1	78.5	
EI	BTM	108	7.7	8.1	0.00
	Other LRCI	63	14.2	10.7	
R index	BTM	108	2.1	0.36	0.00
	Other LRCI	63	3.1	1.1	

Tables 3 and 4 show the parameter and indexes that can be very helpful to the physicians with regard to the detection of thalassemia minor form other types of LRCI anemia. The highest reading of correctly identified patients (PCIP) was reported for RBCs count (84%) in 143 patients. It is positively detected in 104 patients from 108 total patients with TM. The Youden's Index for RBCs was 58.2 which is the highest value compared with other seven parameters or indices which were used in this study. The sensitivity and specificity of RBCs count to detect TM is about 96.3%. The second highest Youden's index was for G & K index (43), with PCIP 78.4%. G & K index was positive in 106 from 108 patients with thalassemia minor with sensitivity and specificity of 98.2%.

Table 3: The differential values of each discrimination index and correctly identified number of patients

Differential values			**Other LRCI**	**BTM**	**TCIP**	**PCIP**
	Other LRCI	**BTM**	**(n=63)**	**(n=108)**	**(n=171)**	**(%)**
RBC $x10^{12}$/L	< 5	> 5	+ 39 - 24	- 4 + 104	143 (39+104)	84

G&K	> 65	< 65	+ 28 - 35	- 2 + 106	134 (28+106)	78.4
R	> 3.3	< 3.3	+ 21 - 42	- 2 + 106	127 (21+106)	74.3
MI	> 13	< 13	+ 32 - 31	- 14 + 94	126 (32+94)	74
KI	>21	<21	+ 19 - 44	- 1 + 107	126 (19+107)	74
RDWI	> 220	< 220	+ 16 - 47	- 1 + 107	123 (16+107)	72
EI	>15	<15	+ 27 - 36	- 19 + 89	116 (27+89)	68
RDW %	>16	<16	+ 18 - 45	- 15 + 93	111 (18+93)	65

TCIP: Total correctly identified patients, PCIP: Percentage of correctly identified patients

Youden's Index of Red cell distribution width (RDW) was the lowest value compared to other values used in this study (15) as well as the lowest percentage of correctly identified patients percent (65%). The sensitivity and specificity of RDW for BTM was 86.1%.

Table 4: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index of each discrimination index

Index		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)	Youden's Indices
RBC $x10^{12}$/L	Other LRCI BTM	62 96.3	96.3 62	91 81.3	81.3 91	84	58.2
G&K	Other LRCI BTM	44.4 98.2	98.2 44.4	93.3 75.2	75.2 93.3	78.4	43
MI	Other LRCI BTM	51 87	87 51	70 75.2	75.2 70	74	38
R	Other LRCI BTM	33.3 98.2	98.2 33.3	91.3 72	72 91.3	74.3	32
KI	Other LRCI BT	30.2 99.1	99.1 30.2	95 71	71 95	74	29.2
EI	Other LRCI BTM	43 82.4	82.4 43	59 71.2	71.2 59	68	25.3
RDWI	Other LRCI BT	25.4 99.1	99.1 25.4	94.1 70	70 94.1	72	25
RDW	Other LRCI BTM	29 86.1	86.1 29	55 67.4	67.4 55	65	15

Hypochromic microcytic anemia, and beta thalassemia minor as a part of it, consists of most common types of anemia in pediatrics, differentiation between beta thalassemia minor and other types of hypochromic microcytic anemia is among the tasks of physicians because each type of diseases has a different cause, treatment and prognosis. Thalassemia is endemic in Iraq because of consanguinity marriage. Misdiagnose of BTT is considered as an important step that increases the incidence of thalassemia diseases in the community. Different equations of blood indices have been used to differentiate causes of hypochromic microcytic anemia using complete blood count parameters especially in low-income countries like Iraq where advance investigation to diagnosis thalassemia syndrome is not always available. No parameter has found 100% sensitivity for thalassemia or other cause of hypochromic microcytic anemia. In this study, we made an attempt to find the best blood indices that help the physicians to be more oriented to BTT early detection in the Iraqi population. In this study, the highest Percentage of correctly identified patients was recorded for red cell count (84%), and Youden's Index of RBCs was 58.2. The Youden's Index of RBCs in thalassemia minor patients in Thailand was

44.6 and in Turkey population was 65.3 [4, 17]. The G & K index was the second highest for Youden's Index (43) with Percentage of correctly identified patients (78.4%). While in a study conducted in India, the Youden's Index of G & K index was 35.5, a study from Greece was the best index of BTT as 65.9 and in a study in Turkey, 56.6 was obtained [17-19]. The MI was the best indicator for BTT screen in a study conducted in India; while, in the current study the Youden's Index of MI was 38 [17, 18].

Shen et al., and Miri-Moghaddam stated that "the spectrum beta-thalassemia mutations in each population can affect various RBC indices. A cut-off value for every formula in different populations should be determined" [20, 21]. The difference in the inter-population differences in the efficiency of methods in the judgment of BTT from IDA could be due to the variations of the molecular spectrum of beta-thalassemic disorders in various countries, the level of anemia in iron-deficient issues, sample range, and the mean age of subjects [22].

CONCLUSION

That automated cell counter-based parameters and formulas are scientifically good, quick, inexpensive, easily available in low-income countries, and reliable for BTT detection compared to other types of hypochromic microcytic anemia. There are no red cells indices, and formulas provided by 100.0% sensitivity, specificity and efficiency for the discrimination of beta thalassemia minor compared to other hypochromic microcytic anemia. According to this study, cell counter-based parameters and formulas, particularly RBCs, Green and King index have good discriminative factor for the screen of BTT status.

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