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Original Article

Evaluation of the Formulae Based on Red Blood Cell Indices in Differentiating Between Iron Deficiency Anemia and Beta Thalasemia Minor

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Background and Aims: Iron deficiency anemia (IDA) and beta thalassemia minor (BTM) are the most common hypochromic microcytic anemias, and it is regarded important to differentiate between them. several formulae can be taken into consideration based on red blood cell (RBC) parameters in order to distinguish between these two disorders. Hence, the present study intended to evaluate the sensitivity as well as specificity of some of these formulae.

Materials and Methods: In this cross-sectional study, IDA was diagnosed in 200 patients based on hypochromic and microcytic RBC appearance, reduced mean cell volume, mean cell hemoglobin and ferritin as well as increased total iron binding capacity. Furthermore, BTM was diagnosed via hypochromic microcytic appearance of RBC and increased hemoglobin A2 level. Then, IDA and BTM diagnosis were confirmed using the formulae of King-Green, Mentzer index, England and Fraser, Shine and Lal, Srivastava and Sirdah. The sensitivity and specificity of these formulae were calculated as well.

Results: The study findings demonstrated that based on the study criteria, out of 200 patients with hypochromic microcytic RBC appearance, 120 were afflicted with IDA and 80 suffered from BTM. The formulae-based diagnosis demonstrated that King-Green formula was the most reliable one.

Conclusions: Although King-Green formula had the highest sensitivity and specificity and was the most reliable formula, none of the formulae revealed 100% sensitivity and specificity. As a result, making definitive distinction between IDA and BTM is not possible using these formulae.

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Introduction

Iron Deficiency Anemia (IDA) is the most common anemia worldwide, which is highly prevalent within Iranian children during their growth, and is a function of iron deficiency for erythropoiesis [1]. Several factors contribute to development of this anemia, among which Iron deficiency in children, gastrointestinal bleeding in male adults and menstrual bleeding in women can be mentioned as the most important reasons [2]. Clinical presentations and laboratory data are regarded beneficial to IDA diagnosis, including reduced mean cell volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb) A2, serum ferritin and increased total iron binding capacity (TIBC). The peripheral blood of these patients shows a hypochromic microcytic appearance [3]. Thalassemia affects nearly 1.5% of global population, which is among the most common single-gene disorders worldwide [4]. It is caused by defective synthesis of globin resulting in decreased production of hemoglobin chains and inherited hemolytic anemia [5]. Depending on the involved chain, various types of thalassemia are caused, including alpha and beta thalassemia. As a matter of fact, based on the involvement degree of beta chain genes, three forms of thalassemia can be mentioned consisting of thalassemia major (Cooley's anemia or Mediterranean anemia), thalassemia intermedia (thalassemia minor) and beta thalassemia carrier (beta thalassemia trait). Thalassemia major is one of the most common single-gene disorders around the world, from which approximately 1.5% of the total population of the developing world suffer [4]. It is caused by a defect in the synthesis of globulin resulting in decreased hemoglobin synthesis and congenital hemolytic anemia [5]. Thalassemia minor is a mild form of thalassemia with defects in only one beta chain, which represents mild symptoms and in some cases no clinical presentations that can be detected via the laboratory findings. The most important laboratory findings entail decreased MCV, MCH, mild reduction in hemoglobin and increased HbA2, among which the latter test is normally time-consuming and expensive. The distinction between this type of thalassemia and IDA is generally based on HbA2 measurement and mutation presence in the beta-globulin gene. Several formulae can be taken into account based on red blood cell indices aiding the differentiation between IDA and thalassemia minor. The sensitivity and specificity of some of the formulae have been investigated in this study demonstrated in table 1.

Materials and Methods

This cross-sectional study consisted of 200 patients suffering from IDA (n=120) and thalassemia minor (n=80). The IDA criteria included hypochromic-microcytic anemia with reduced MCV and MCH as well as biochemical tests results such as reduced serum ferritin levels below 12 ng/dl and TIBC higher than 400 µg/dl. Hypochromic-microcytic anemia with reduced MCV and MCH along with

HbA2>3.5% was considered in order to diagnose BTM. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden's index of formulae listed in table 1 were calculated to differentiate between IDA and BTM as follows:

$$sensitivity = \left[\frac{true\ positive}{(true\ positive + false\ negative)}\right] \times 100$$

$$specivity - \left[\frac{true\ negative}{(true\ negative + false\ positive)}\right] \times 100$$

$$PPV = \left[\frac{true\ positive}{(true\ positive + false\ positive)}\right] \times 100$$

$$NPV = \left[\frac{true\ negative}{(true\ negative\ |\ false\ negative)}\right] \times 100$$

$$Youden's\ index\ =\ (sensitivity\ +\ specificity)\ -\ 100$$

Table 1. Formulae contributing to the differentiation between thalassemia minor and IDA

Hematological Index	Formula
King-Green(K and G)	MCV×RDW/Hb×100
Mentzer index (MI)	MCV/RBC
England and Fraser (E and F)	MCV− (5 × Hb) −RBC−3.4
Shine and Lal (S and l)	MCV×MCV×MCH/100
Srivastava	MCH/RBC
Sirdah	MCV− RBC− (3×Hb)

Results

Hematological parameters as well as indices of IDA and BTA patients are shown in table 2. Based on the diagnostic criteria, out of a total of 200 patients, 120 were reported to have IDA and 80 were observed to suffer from BTM. IDA and BTM were diagnosed based on the formulae of King and Green (K and G), Mentzer index (MI), England and Fraser (E and F), Shine and Lal (S and L), Srivastava and Sirdah. Furthermore, accurate diagnosis ratio of IDA and BMT patients was calculated as well (Table 3).

Table 2. Hematological and biochemical parameters in IDA and BMT patients

	BTM Mean	IDA Mean
RBC (10^6 /L)	5.8	4.87
Hb (gr/dl)	10.9	11.3
MCV (fl)	62.3	68.1
MCH (Pg)	19.9	22.4
MCHC (gr/dl)	29.4	31.2
RDW (%)	16.3	17.2
SI (µg/dl)	72	25.2
TIBC (µg/dl)	375.2	468.5
Ferritin (ng/ml)	41	11.2

Table 3. IDA and BMT diagnosis according to the formulae

	IDA (120)	B-thal(80)	Correctly diagnosed(n)	Correctly diagnosed(%)
King-Green				
IDA>13	119	4	195	97.5
B-Thal<13	1	76		
Mentzer				
IDA>13	118	6	192	96
B-Thal<13	2	74		
England-Fraser				
IDA>0	115	5	190	95
B-Thal<0	5	75		
Sirdah				
IDA>27	112	8	184	92
B-Thal<27	8	72		
Shain and lal				
IDA>1530	107	15	172	86
B-Thal<1530	13	65		
Sirvastava				
IDA>3.8	110	9	181	90.5
B-Thal<3.8	10	71		

Table 4. Sensitivity, specificity, PPV, NPV and Youden's index results of the formulae

	Sensivity	Spcifity	PPV	NPV	Youden's index
King-Green					
IDA	99.16	95.0	96.74	98.70	94.16
B-Thal	95.0	99.16	98.70	96.74	
Mentzer					
IDA	98.33	92.50	95.16	97.36	90.83
B-Thal	92.50	98.33	97.36	95.16	
England-Fraser					
IDA	95.83	93.75	95.83	93.75	89.58
B-Thal	93.75	95.83	93.75	95.83	
Sirdah					
IDA	93.33	90.00	93.33	90.00	83.33
B-Thal	90.00	93.33	90.00	93.33	
Shain and lal					
IDA	89.16	81.25	87.70	83.33	70.41
B-Thal	81.25	89.16	83.33	87.70	

Discussion

Red blood cells demonstrate a hypochromic microcytic appearance in IDA and BTM. It is not possible to differentiate between them only via red blood cell parameters. HbA2 measurement as well as mutation in beta globin gene can be regarded as other procedures in order to distinguish between IDA and BTM. Since these tests are time-consuming and costly, several formulae have been developed based on RBC parameters in order to distinguish between IDA and BTM. In the current study, IDA and BTM were first diagnosed according to hematological parameters as well as biochemical tests, and then sensitivity and specificity of King-Green, Mentzer index, England and Fraser, Shine and Lal, Srivastava and Sirdah formulae were investigated.

In this study, King-Green formula was reported to have the highest sensitivity and specificity, which as a result, was the most reliable formula followed by Mentzer formula. In line with the findings of the present study, many other studies have also correlated the sensitivity and specificity of various formulae, and none displayed 100% specificity and sensitivity. Ntios et al. (2007) proposed that King-Green formula was the most reliable formula to distinguish between IDA and BTM [6]. In another study, Miri-Moghaddam et al. (2014) showed similar results as well [7], that the results of both mentioned studies were consistent with those of the present study. Batebi et al. (2012) evaluated the sensitivity and specificity of Mentzer, Shine & Lal, and England & Fraser formulae, who indicated the highest specificity and sensitivity for the England & Fraser formula [8]. Their results were not in line with those of this study which may lie in the fact that the King-Green formula had not been evaluated. Based on the findings of the current study as well as previous studies, it can be concluded that King-Green formula is the most reliable formula to differentiate between IDA and BTM, although none of the formulae are 100% reliable.

Conclusion

The findings of the present study proposed that applying some formulae can be considered fruitful in the differentiation between the IDA and BTM, among which the King and Green formula were reported to have the highest sensitivity.

Conflict of Interest

The authors declare that they have no conflict of interests in this work.

Acknowledgement

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References

[1]. Konno M, Toita N, Yokota S. [Iron-deficiency anemia]. Nihon rinsho Japanese journal of clinical medicine. 2013;71(8):1462-466.

[2]. Naigamwalla DZ, Webb JA, Giger U. Iron deficiency anemia. The Canadian Veterinary Journal 2012;53(3):250.

[3]. Punnonen K. Laboratory diagnosis of iron deficiency anaemia. Scandinavian journal of clinical and laboratory investigation 2005;65(7):533-34.

[4]. Galanello R, Origa R. Review: beta-thalassemia. Orphanet J Rare Dis. 2010;5(11).

[5]. Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. Blood reviews 2008;22(5):283-92.

[6]. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening tests for β-thalassemic trait. Annals of hematology 2007;86(7):487-91.

[7]. Miri-Moghaddam E, Sargolzaie N. Cut off determination of discrimination indices in differential diagnosis between iron deficiency anemia and β-thalassemia minor. International journal of hematology-oncology and stem cell research 2014;8(2):27-32.

[8]. Batebi A, Pourreza A, Esmailian R. Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices. Turk J Med Sci. 2012;42(2):275-80.