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Most Reliable Haematological Indices for Diagnosis of Iron Deficiency Anaemia from Non-Iron Deficiency Anaemia in Reproductive-Age Females

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ABSTRACT

Iron deficiency anaemia has become a major public health issue, especially in women of reproductive age. About 50% of anaemia is caused by iron deficiency anaemia. Numerous statistical procedures based on red blood cell parameters have been developed to easily, quickly, and inexpensively provide a differential diagnosis for the microcytic anaemia. The study aims to evaluate the reliability of the haematological discrimination indices (RBC count, Mentzer index, Red blood cell distribution width index (RDWI), Srivastava index, Green and King index, and Ehsani index) to distinguish iron deficiency anaemia from other causes of the microcytic anaemia. The blood samples were collected from non-pregnant women, and they had the microcytic anaemia. During the study, 101 blood samples were taken. A Complete blood count test and serum ferritin test were performed to calculation of the sensitivity, specificity, positive, and negative predictive value for each discrimination indices. The validity of discrimination indices was evaluated by using Youden's index. In correctly distinguishing iron deficiency anaemia from other causes of the microcytic anaemia, the Green and King index and RBC count showed the highest ability (77%) and (76%), respectively. None of the discrimination indices had 100% sensitivity or specificity. The highest and lowest sensitivity was found for the RBC count and RDWI, at 92% and 70%, respectively. The RDWI had the highest level of specificity (65%), and the Srivastava index had the lowest level of specificity (30%). The highest positive predictive value found for both Green and King, and RDWI was 78%, and the RBC count had the highest negative predictive value (78%). The Srivastava index had the lowest levels of positive and negative predictive values, 67% and 50%, respectively. According to the findings of this study, the most reliable index in the calculation of Youden's index is the Green and King index. It can be used as a pre-diagnostic tool for IDA and other causes of the microcytic anaemia.

Keywords: Microcytic anaemia; Iron deficiency anaemia; Non-pregnant women; Haematological index; Iraq.

1. Introduction

Anaemia occurs when the Haemoglobin (Hgb) concentration in red blood cells falls below a certain cut-off value, decreasing in the blood's ability to carry oxygen throughout the body[1]. About 50% of anaemia in women worldwide is caused by iron deficiency[2]. When the body does not have enough iron to support normal physiological functioning, an iron deficiency can develop. Iron deficiency can be recognized by a drop in serum ferritin or total body iron. Iron deficiency anaemia (IDA) develops if bodily iron levels drop too low to maintain healthy RBC synthesis[3]. The serum ferritin test is the most common and useful biochemical test that most closely reflects the relative stored body iron. When the infection is absent, a low serum ferritin degree suggests that the body's iron stores have been reduced, which is a precondition closer to iron deficiency[4]. There are many different causes of anaemia, several of which can exist alone but more frequently interact.

A series of essays is necessary to differentiate IDA, including thorough peripheral blood analysis, serum ferritin, transferrin saturation, serum iron, and total iron binding capacity[5]. Although, each of these tests mentioned is either not available in most clinical settings or requires extremely time-consuming and expensive procedures[6]. Various discriminating indices have been used in haematology, like the Green and King, Red cell distribution width (RDW), England and Fraser, RBC count, Shine and Lal, Ricerca, Mentzer, Ehsani, Sirdah, Huber Herklotz, and

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Srivastava indices. None of them has a sensitivity or specificity of 100%. All of these indices are derived from various haematological criteria, such as RBC count, Hgb, Mean cell volume (MCV), and RDW, because the automated blood cell analyzers can rapidly and easily give these blood parameters, even in a laboratory that does not contain much equipment[7]. This study aims to evaluate the reliability of six haematological discriminate indices to distinguish iron deficiency anaemia from other causes of the microcytic anaemia (non-iron deficiency anaemia) in non-pregnant women living in Garmian province, Kurdistan region.

2. Methods and Materials

2.1 Study setting

The study was conducted in the city of Kalar in the Garmian province-Iraqi Kurdistan region. After obtaining agreement from each patient to participate in this study. More information was collected from each patient by filling out a prepared study form. The information included their place of residence, age, and whether she was pregnant or not. During the study, 101 blood samples were collected from non-pregnant females between the ages of 16–49 years, who had microcytic anaemia, resided in Garmian province, and had visited both Garmian Medical City and Zanko Medical Laboratory from the period August 2021–April 2022.

2.2 Laboratory work

During the study, after cleaning the site of drawing the blood sample with a remarkable antiseptic (%75 alcohol), 5 milliliters of blood were taken, and patient's name and date of the collection were recorded. Based on the results obtained from the CBC test, each of the haematological discrimination indices was calculated. After that, a serum ferritin test was done for the detection and reliability of the haematological discrimination indices by calculating of the sensitivity, specificity, true positive, and false negative values.

2.2.1 Complete Blood Count test

For the performance of the Complete blood count (CBC) test, the collected blood was stored in a special tube containing anticoagulant EDTA, and then the CBC test was performed immediately. The CBC test gives specific information about red blood cells, haemoglobin, hematocrit, mean corpuscular volume, mean cell haemoglobin, mean cell haemoglobin concentration, and red blood cell distribution width. In the Zanko Medical Laboratory, the CBC test was analyzed by a haematology analyzer (Medionic M51, made in Sweden), and the blood samples were collected in Garmian Medical City and analyzed by an automated haematology analyzer (Swelab Alfa Plus, made in Sweden).

2.2.2. Serum Ferritin test

For the performance of the serum ferritin test, the blood was stored in a special tube containing gel and clot activator. Then for separation of serum from blood elements, the blood was centrifuged at 3500 rounds per minute (rpm) for 10 minutes by (LC-04L Centrifuge, made in China) and (80-1 Electric Centrifuge, made in China) in the Zanko Medical Laboratory and Garmian Medical City, respectively. The serum ferritin test was assessed by a (Cobas e411 analyzer, made in Japan) with the serum ferritin kit (Elecsys Ferritin, REF 03737551190, M 6.5 mL, R1 and R2 10 mL, made by Roche Diagnostics GmbH).

2.3 Differential value and statistical analysis

For the diagnosis of iron deficiency anaemia, WHO guidelines were used. According to the WHO guidelines, non-pregnant women who measured Hgb<12 g/dl, and serum ferritin <15 µg/L were defined as IDA[4]. Patients with MCV<80 fl are considered microcytic[8]. The differential values for each of the haematological discrimination indices used in the assessment of IDA were applied by the previously described study as follows: RBC count, Mentzer index (MI); (MCV/RBC), RDW index (RDWI); (MCV × RDW/RBC), Srivastava index (SI); (MCH/RBC), Green and King index (G and KI); (MCV × MCV × RDW/Hb × 100)[9], and Ehsani index (EI); (MCV − (10 × RBC))[10].

For each haematological discrimination indices, previously proposed formulas were used to compute the sensitivity, specificity, positive predictive value, and negative predictive value. They were calculated as follows:

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

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

Positive Predictive Value (PPV)= true positive /(true positive+false positive)×100

Negative Predictive Value (NPV)= true negative/(true negative+false negative)×100

Youden's index= (Sensitivity+ Specificity-100) [9].

The *t*-test was used to compare parameters between IDA and other causes of the microcytic anaemia (non-IDA) (Table 1). *P*-value <0.05 was considered statistically significant[7].

2.4 Ethical Consideration

The Ethical approval letter was obtained from the scientific and ethical committee of the Department of Biology, College of Education, and the University of Garmian. All patients provided informed consent to participate in this study; each participant was informed about the objectives of this study.

3. Results

3.1 Haematological and Biochemical values

From the collected microcytic blood samples, 64 patients had IDA and 37 patients with other causes of the microcytic anaemia (who had not been diagnosed with IDA). The mean of RBC, Hgb, MCV, MCH, MCHC, RDW, and serum ferritin differed in both IDA and non-IDA patients (Table 1). In these parameters, RBC, Hgb, Hct, MCH, and serum ferritin of the non-IDA patients were higher than those of the IDA patients. The MCV, MCHC, and RDW were comparable in both IDA and non-IDA patients. RBC, Hgb, Hct, and serum ferritin were significantly differed between IDA and non-IDA patients (*P*<0.0001). The results also showed that the MCV, MCH, MCHC, and %RDW were no significant differences between the IDA and non-IDA patients (*P*>05).

Table 1: Haematological and biochemical parameters obtained from the screened patients.

Haematological and biochemical parameters	IDA		non-IDA		*t*-test	
	Mean	SD	Mean	SD	*t*-statics	*P*-Value
RBC ($\times 10^{12}$/L)	4.4	0.5	5.1	0.54	5.809	0.00001*
Hgb (g/dL)	9.2	1.5	10.6	0.75	6.276	0.00001*
Hct (%)	30.2	4.34	34.3	2.07	6.375	0.00001*
MCV (fl)	68.4	8.37	68.2	6.64	-0.109	0.913
MCH (pg)	20.8	1.33	21.1	1.98	0.635	0.526
MCHC (g/dL)	30.3	1.33	30.6	2.06	0.784	0.436
%RDW	15.6	2.37	15.5	3.22	-0.109	0.913
SF	6.1	3.26	94.9	128.35	4.225	0.0001*

RBC: Red Blood Cells; Hgb: Haemoglobin; Hct: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Haemoglobin Concentration; RDW: Red cell Distribution Width; IDA: Iron Deficiency Anaemia; Non-IDA: non-Iron Deficiency Anaemia; SD: Standard Deviation; *A P-value of <0.05 is considered significant

3.2 Correctly distinguishing IDA

As indicated in table 2 and figure 1, of the total sample, the G and KI showed the ability of (77%) and the RBC count of (76%), which is the highest ability in correctly distinguishing IDA from other causes of the microcytic anaemia, followed by RDWI, MI, EI, and SI (68%), (67%), (66%), and (63%) of the total sample, respectively. None_of the evaluated indices provided 100% accuracy in identifying IDA.

Table 2: The ability of each haematological discrimination index to correctly distinguish iron deficiency anaemia from other causes of microcytic anaemia (n=101).

Haematological indices	Differential value	IDA (n=64)	non-IDA (n=37)	Total No. of correctly Identified (Sens+Spec)	Percentage of Correctly identified (%)
RBC count	IDA< 5	59	18	77 (59+18)	76
Mentzer	IDA> 13	51	17	68 (51+17)	67
RDWI	IDA> 220	45	24	69 (45+24)	68
Srivastava	IDA> 3.8	53	11	64 (53+11)	63
Green & King	IDA> 65	57	21	78 (57+21)	77
Ehsani	IDA> 15	50	17	67 (50+17)	66

IDA: Iron Deficiency Anaemia; non-IDA: non-Iron Deficiency Anaemia; Sens: Sensitivity; Spec: Specificity

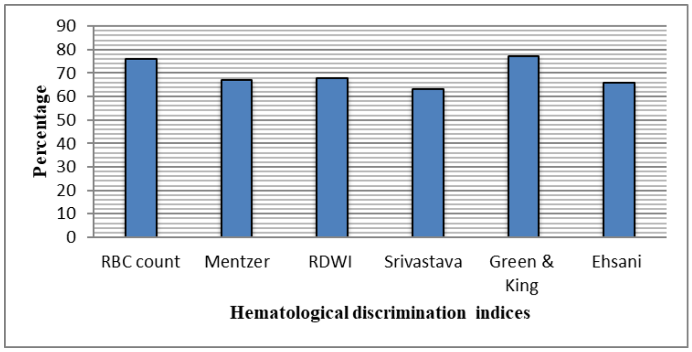

Figure 1: The percentage of the ability of each haematological discrimination index to correctly distinguish iron deficiency anaemia from other causes of microcytic anaemia.

3.3 Sensitivity, specificity, positive, and negative predictive, and Youden's index

The values of sensitivity, specificity, positive and negative predictive values, and Youden's index are shown in table 3 and figure 2 for all the discrimination indices which are used in the diagnosis of IDA. For identifying IDA, the RBC counts compared with the other indices, showed the highest sensitivity (92%), In comparison, the RDWI had the lowest sensitivity (70%), followed by the indices G and KI, SI, MI, and EI, which had those values of (89%), (83%), (80%), and (78%), respectively. While the RDWI showed the highest specificity (65%), whereas the SI had the lowest value of specificity (30%), the G and KI showed specificity (57%), the RBC count (49%), after the above mentioned, the MI and EI come, that showed the same specificity (46%). Furthermore, table 3 and figure 2 show the highest and lowest PPV and NPV. The highest value PPV value was found for both G and KI, RDWI (78%), and the RBC count had the highest rate of NPV value (78%). SI had the lowest PPV value (67%) and NPV value (50%). Moreover, the highest and lowest values of Youden's index are displayed in table 3 and figure 3, in which the G and KI provided the highest level (46), still the SI provided the lowest level (13), followed by the RBC count, RDWI, MI, and EI (41),(35), (26), and (24), respectively.

Table 3: Sensitivity, specificity, positive predictive value, and negative predictive value (NPV), and Youden's index of each haematological discrimination index in the differentiation of IDA from other causes of microcytic anaemia.

Haematological indices	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Youden's index
RBC count	92	49	76	78	41
Mentzer	80	46	72	57	26
RDWI	70	65	78	56	35
Srivastava	83	30	67	50	13
Green and King	89	57	78	75	46
Ehsani	78	46	71	55	24

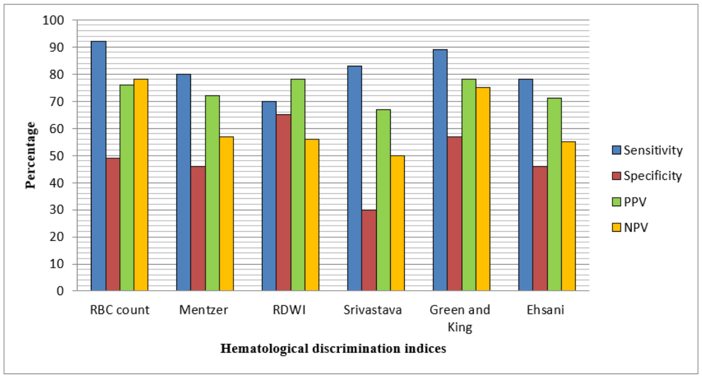

Figure 2: The percentage of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each haematological discrimination index to distinguish iron deficiency anaemia from other causes of microcytic anaemia.

of the indices could identify IDA with 100% sensitivity or specificity. The G and KI showed great sensitivity, specificity, and the best level of Youden's index value (89 %), (57%), and (46), respectively. The RBC count showed the greatest sensitivity value, and displayed second-high values in Youden's index and low specificity compared to the GI and KI. According to the rating of the index's capacity to detect IDA, Youden's index revealed the following ranking: G and KI > RBC count > RDWI > MI > EI > SI.

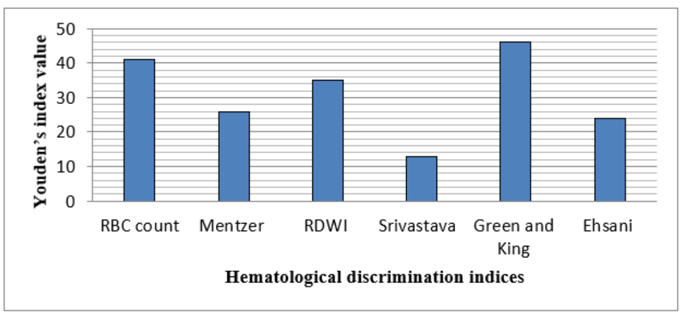

Figure 3: The Youden's index value of each evaluated haematological discrimination index.

4. Discussion

Iron deficiency anaemia has become a major public health issue, especially in women of reproductive age. It affects women's health and well-being and is linked to poor reproductive outcomes[11]. The most prominent etiologic of the microcytic anaemia is iron deficiency. Menorrhagia and long-term blood loss without the appropriate iron supplements, it usually happen in premenopausal and menstrual women[8]. The distinction of IDA from other causes of microcytic anaemia (especially the Beta Thalassemia Trait) has a very significant clinical impact due to each of these diseases having completely different causes, prognostics, and methods of treatment[12].

In the past, these haematological discriminations indices evaluated in this study had not been used as a diagnostic tool for IDA or other causes of the microcytic anaemia in our region. In the present study, six discrimination indices have been calculated to correctly determine the ability of each of them in the diagnosis of IDA. Among the results obtained in this study, none of the discrimination indices could identify IDA with 100% sensitivity and specificity, similar to the results reported by many researchers[5, 9, 12-14]. The sensitivity of all studied discriminant indices was recorded as a high value compared with the specificity of discriminant indices for the diagnosis of IDA. These results were in contrast with those of the study by Vehapoglu et al.[12], they evaluated 12 haematological discrimination indices, including the Mentzer index, RDWI, RBC count, Ehsani index, Green and King index, and Srivastava index, to differentiate iron deficiency anaemia from beta thalassemia trait. According to their findings, 11 of the haematological indices exhibited lower sensitivity values than specificity values for distinguishing iron deficiency anaemia, and our results were similar to those of the study by AlFadhli et al.[15]. The RBC count in our study had the highest sensitivity (92%), followed by the Green and King index, Srivastava index, Mentzer index, and Ehsani index, However, the RBC count showed a specificity of 49%, and during the diagnosis time, in the 101 studied non-pregnant women with the microcytic anaemia, (77%) of them had a low to normal RBC count (RBC count <$5x10^{12}$/L) and (23%) of them had a high RBC count (RBC count >$5x10^{12}$/L). The sensitivity and specificity of RBC count were (96%) and (86 %) in the study of Demir et al.[9]; (70.5%) and (94.8%) in the study of Vehapoglu et al.[12]; (86%) and (90%) in the study of Jameel et al. [6]. According to our results, the RBC count is regarded as an important diagnostic tool for IDA. Haematopoiesis is a process that produces RBCs, and uses iron as a component of Hgb [16]. When IDA is present, low RBC count and low MCV result from the bone marrow's inability to manufacture enough RBCs and their smaller size[17].

According to our results, the RDWI showed the highest value of specificity (64.86%), followed by the G and KI, RBC count, EI, MI, and SI. Demir et al.[9] compared eight discriminating indices; the RDWI showed the greatest specificity (100%) compared with all the studied indices. The RDWI is considered one of the most favourable indices due to the inclusion of discriminating variables such as RBC count, MCV, and RDW in its formula [14]. In the obtained results of the present study, the G and KI exhibited sensitivity and specificity of 89% and 57%, respectively. In comparison, the MI index showed sensitivity and specificity of 80% and 46%, respectively, in the demonstration of IDA. The results are comparable to those of Rastogi and Bhake[18]. They found these to be 68% and 89.74%, respectively, for G and KI, and 85.75% and 94.87% for MI.

In our results, the G and KI had the highest values for correctly diagnosing IDA from other causes of the microcytic anaemia, and the SI index showed the lowest value, as it appeared the results of these indices have the ability 63-77% to identify patients correctly with the microcytic anaemia. Rahim and Keikhaei[19] showed the ability of the discrimination indices to correctly identify patients. The ability of the discrimination indices in their study ranged from 67-93%. Additionally, the discrimination indices of the study of Pornprasert et al.[20] to correctly distinguish IDA and BTT showed the ranking for our haematological indices, which were used in our study; SI > EI > MI > RBC count > G and KI > RDWI. A summary of these comparisons is shown in table 4.

Table 4: Summary of the previous studies that demonstrated the ability of the discrimination indices to distinguish iron deficiency anemia from thalassemia correctly.

References of the previous study	Haematological indices					
	RBC count (%)	Mentzer (%)	RDWI (%)	Srivastava (%)	Green and King (%)	Ehsani (%)
[20]	59.7	94.8	36.4	97.4	51.9	96.1
[9]	90	76	92	67	86	-
[12]	83.4	91	80	79.3	78.6	84.8
[10]	92.61	94.71	-	86.97	-	-
[19]	90	89	93	84	67	-
[24]	82.95	86.05	92.25	82.17	92.25	86.05

Youden's index provides the most suitable standard of validity for a particular subject or method, which incorporates both sensitivity and specificity. According to the results of Youden’s index value, the most reliable indices were the G and KI, which recorded the highest value, followed by RBC count, RDWI, MI, EI, and SI, respectively. The study by Shen et al.[7] showed that the G and KI, and England and Fraser index had high validity in diagnosing of IDA from BTT. Furthermore, G and KI were calculated as the most reliable index in the diagnosis of IDA from BTT in many studies[13, 21]. AlFadhli et al.[15] evaluated nine discriminant functions and assessed their validity using Youden's index. They demonstrated that the Shine and Lal’s index is ineffectual at identifying the microcytic anaemia with the Youden index. In contrast, the England and Fraser index showed the greatest Youden index value for accurately distinguishing IDA in patients with the microcytic anaemia. According to another study, Youden’s index of RBC count and RDWI were the highest, with a value of 82 and 80, respectively. In contrast, Shine and Lal's index exhibited the lowest Youden’s index value (zero)[9]. Vehapoglu et al.[12] measured the validity of each discriminant indices and compared several haematological discriminant indices. They showed that the MI had the highest value of Youden’s index (81), while the MCHD index had the lowest Youden’s index value (5.8). Okan et al.[22] suggested that the G and KI, and Shine and Lal index is the best discriminator to demonstrate the microcytic anaemia due to IDA or BTT. In the study of Beyan et al.[23] the sensitivity, specificity, PPV and NPP, and Youden’s index, have been determined for nine discriminant haematological indices in Turkey. In their study, the G and KI recorded the second-highest Youden index value (65.5), while the Ricerca index had the lowest Youden index value (-0.2). A summary of these comparisons is shown in table 5.

Table 5: A summary of the comparisons.

References of the previous study	Haematological indices					
	RBC count	Mentzer	RDWI	Srivastava	Green and King	Ehsani
[25]	63	56.9	56.2	-	64	-
[26]	-12.5	41.4	27.9	45.5	26.1	-
[23]	73.7	64.6	63.4	50.2	65.5	-
[21]	-	83.2	84.18	80.65	84.3	-
[12]	65.3	81	59.5	57.7	56.5	68.3
[24]	67.76	74.34	82.76	64.13	82.76	75.13
[20]	44.6	92.9	12.5	96.4	33.9	94.6

Finally, none of these various formulations is better than the Green and King index depending on Youden’s index values. It can be used as a pre-diagnostic tool for IDA from other types of the microcytic anaemia. Until more effective methods are developed, serum ferritin, serum iron, transferrin saturation, and total iron binding capacity should be measured for reliable differential diagnosis of IDA. The abovementioned studies are required for everyone with low MCV and Hgb.

Conclusion

These discrimination indices evaluated in the present study had a great ability to diagnose IDA, but none of them had perfect sensitivity or specificity. According to the finding of this study, the most reliable index in the calculation of Youden's index (sensitivity+specificity-100) is the Green and King index.

Recommendation

The Green and King formula can be used as an initial diagnostic tool for iron deficiency anaemia in areas lacking facilities for serum ferritin, serum iron, total iron binding capacity, and Hgb electrophoresis tests.

In Future

In our future study, we will develop three haematological indices specific to the researchers of this study for the diagnosis of IDA from other causes of the microcytic anaemia that can diagnose the disease at a very high level.

Conflict of interests

None

Contribution

All authors of this study participated equally in all stages of the writing, review and submission of this study.

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