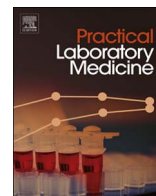




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Should sex differences be considered when applying mathematical indices and formulas for discriminating β -thalassemia minor from iron deficiency?

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ABSTRACT

Background: β -thalassemia minor (BTM) and iron deficiency (ID) are common disorders characterized by microcytosis and/or hypochromasia, leading to a challenge in their discrimination during mass-screening programs especially in developing countries where resources are limited. It has been shown with varying reliability that quick exclusion of either disorder could be achieved mathematically using RBC-based indices and formulas. However, none of these proposed indices and formulas considered the sex-based hematological differences. This comparative retrospective study examined the efficacy of using sex-based RBC indices in the mathematical discrimination BTM and ID in adult males and females.

Methods: The CBC of randomly selected eight hundred adults diagnosed with BTM or ID (200M & 200F BTM, and 200M & 200F ID) were used in the comparisons. The discrimination power, in terms of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and Youden index were calculated for all subjects and separately for males and females for 20 mathematical indices and formulas.

Results: Data revealed significant differences in the RBC-based indices between males and females for both BTM and ID groups. Significant variation in reliability indicators for the different indices and formulas were discovered between males and females samples.

Conclusion: Sex-based indices and formulas are necessary to improve the reliability in mathematically discriminating between BTM and ID in mass screening programs. We also advocate for a large-scale multicenter study to establish the parameters of such indices and formulas with sex and age.

1. Introduction

Although the clinical diagnosis of individuals with BTM or ID must be made through a variety of different confirmatory laboratory tests, such as quantitation of HbA₂ percentage and serum ferritin levels [1,2], however, the availability of these tests could be limited for mass-screening programs in developing countries where resources are scarce. Since the early 1970's there has been a great interest to find an easy, quick and inexpensive approach to differentiate between carriers of β -thalassemia (BTM) and iron deficiency (ID) as

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both display similar symptomatic profiles (microcytic and/or hypochromic blood). With the development of automated hematological analyzers, the complete blood count (CBC) has become a quick and reliable test that can provide valuable hematological information to physicians, leading to provisional diagnosis and /or to direct further testing. Many approaches using different mathematical indices and formulas based on routine CBC parameters have been proposed as quick and simple indices and formulas to differentiate a blood sample as BTM or ID. Most of these mathematical indices and formulas included one or a combination of key CBC parameters in the differentiation primarily the RBC, MCV, Hb and RDW. The England and Frazer formula ($MCV - RBC - 5 \times Hb - 3.4$), Mentzer formula (MCV/RBC) and Srivastava formula of the 1973 were some of the earliest attempts to mathematically characterize BTM and ID blood samples [3–5].

Over the past 50 years a substantial number of studies in different populations attempted to evaluate these formulas or even generate and suggest new ones aimed at finding the most reliable indices or formulas with the highest sensitivity and specificity or predictive value [6–9]. However, none of the many different indices or formulas provided 100% sensitivity and 100% specificity within their target populations nor was one formula superior to another, within or between populations. Such inter-populations variation in discriminating formulas could be attributed to differences in the mutation spectrum of thalassemia disease in different populations [10]. Nevertheless, none of these indices and formulas were addressed in terms of the sex-based differences of RBC parameters and indices. Therefore, we explored the possibility that sex-based RBC indices and formulas could provide superior discrimination between BTM and ID in adult males and females separately as compared to the both sexes being considered as one group.

2. Materials and methods

The present study included the complete blood count (CBC) results of randomly selected eight hundred adults (200 males and 200 females with BTM, and 200 males and 200 females with ID). The CBC including counts of white blood cells (WBC), red blood cells (RBC), platelets (PLT) and hemoglobin (Hb) concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) was performed using a Cell Dye 1700 electronic counter (Sequoia-Turner Corporation, California, USA).

The adult (age 18–40 years old) samples were randomly selected from the subjects of the premarital tests screening program of β -thalassemia in Gaza Strip, which was launched as obligatory testing since September 2000 under full control and supervision of the Thalassemia Center, Palestine Avenir Foundation. All included subjects were apparently healthy adults. Cases of chronic diseases were primarily excluded, as they are not eligible for the study inclusion criteria. It is worthwhile mentioning that the premarital tests are being performed according to a defined working protocol in accordance with the ethical standards laid down in the 1964 and 1975 Declarations of Helsinki, and the modifications thereafter. The protocol has been officially approved by the scientific and ethical committees at the Thalassemia and Hemophilia Center, Palestine Avenir Foundation.

Individuals were selected retrospectively, based on low MCV ($MCV < 80$ fl) and MCH ($MCH < 26$ pg) values which suggested microcytosis and/or hypochromasia, two common features of blood samples from BTM or ID subjects. According to the working protocol of the premarital tests screening program, diagnosis of the microcytic and /or hypochromic suspicious cases was confirmed by laboratory testing including hemoglobin subtype electrophoresis; chromatographic quantitative determination of HbA_2 , and measurement of serum ferritin levels using an IMX automated immunoassay analyzer system (Abbott, Abbott Park, IL, USA). All microcytic and/or hypochromic cases were classified as BTM if their $HbA_2 > 3.5\%$, and as ID if their serum ferritin level < 12 μ g/dl [11,12]. Any records of combined cases (BTM with ID) were preliminary excluded from the random selection of the samples.

We evaluated the discrimination power (in terms of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, Youden index; positive and negative predictive values) for males, females and both for the following mentioned 20 indices and formulas Table 1. We also constructed the receiver operative characteristic (ROC) curve and calculated the area under the curve (AUC) for each index and formula and associated these with their original cut-off discrimination values for BTM.

Although some studies suggested revised cut-off values only the original cut-off values, first generated by their authors, were used in the study. All subject data was encoded and analyzed using IBM SPSS Statistics (version 20, IBM Corporation, Somers, NY).

3. Results

The population of this study comprised microcytic and/or hypochromic CBC results retrospectively selected from the β -thalassemia center database for obligatory premarital testing conducted in the Gaza Strip according to defined and approved protocols. From the total of about 18,000 microcytic and/or hypochromic CBC results, we randomly selected 800 cases into two groups. The first group included 400 BTM cases (200 males and 200 females) whose diagnosis was confirmed by their $HbA_2 > 3.5\%$. The second group included 400 ID cases (200 males with 200 females) where the diagnosis was confirmed by serum ferritin level < 12 μ g/dl. The RBC parameters of both groups (BTM and ID) as well as the sex-based differences are listed in Table 2. ANOVA analysis revealed significant differences ($p < .001$) between the groups (BTM and ID), as well as, sex subgroups in all RBC-based parameters except for MCHC ($p = 0.091$) and RDW ($p = 0.789$).

We evaluated efficiency (Table 3) in terms of correctly diagnosed cases for index and formula for males and females separately and then as one group. Sex-based differences in efficiencies were reported for most the discrimination indices and formulas. One recognizable observation is an inverted efficiency trend of most indices and formulas. Wherein, an index or formula that provided a high efficiency for BTM and low efficiency for ID in one sex provided a high efficiency for ID and low efficiency for BTM in the other sex (Fig. 1). For example, England and Fraser Formula provided an efficiency of 93.0% and 64.0% in detecting BTM and ID

Table 1The twenty mathematical indices and formula that used for the discrimination of β -thalassemia minor.

Discrimination index or formula	Cut-off value	Reference
Bordbar formula: $(80-MCV \times 27-MCH)$	> 44.76	[13]
Ehsani Formula: $(MCV-10 \times RBC)$	< 15	[14]
England and Fraser formula: $(MCV-RBC-5 \times Hb-3.4)$	< 0.0	[3]
Green and King Formula: $(MCV^2 \times RDW/Hb \times 100)$	< 72	[15]
Huber-Herklotz Index: $(MCH \times RDW \times 0.1/RBC) + RDW$	< 22	[16]
Kandhro index #1: $(RBC/HCT + 0.5 \times RDW)$	< 8.2	[8]
Kandhro index #2: $(RDW \times 5)/RBC$	< 16.8	[8]
Keikhaei index: $Hb \times RDW \times 100 / (RBC)^2 \times MCHC$	< 21	[17]
Kerman Formula #1: $MCV \times MCH/RBC$	< 300	[18]
Kerman Formula #2: $(MCV \times MCH/RBC) \times 10/MCHC$	< 85	[18]
Matos-Carvalho Index: $(1.91 \times RBC) + (0.44 \times MCHC)$	> 23.85	[19]
Mentzer formula: (MCV/RBC)	< 13	[4]
RDW index: $(RDW \times MCV)/RBC$	< 220	[20]
Ricerca index: (RDW/RBC)	< 3.3	[20]
Shine - Lal Formula: $(MCV^2 \times MCH/100)$	< 1530	[21]
Sirachainan index: $1.5 \times Hb - 0.05 MCV$	> 14	[22]
Sirdah Formula: $MCV-RBC-3 \times Hb$	< 27	[10]
Srivastava formula: (MCV/RBC)	< 3.8	[5]
Zaghloul index #1: $Hb + Hct + RBC$	Male & females > 52.5 Males > 55.7 Females > 51.6	[7]
Zaghloul index #2: $Hb + Hct + RBC - RDW$	Male & females > 37.1 Males > 40.4 Females > 35.6	[7]

Table 2mean \pm standard deviation of RBC-related parameters of the overall, BTM and ID subjects as well as sex subgroups.

Index	Overall $n = 800$	BTM			ID		
		Males $n = 200$	Females $n = 200$	Overall $n = 400$	Males $n = 200$	Females $n = 200$	Overall $n = 400$
RBC	5.35 \pm 0.70	6.12 \pm 0.48	5.32 \pm 0.43	5.72 \pm 0.61	5.34 \pm 0.52	4.61 \pm 0.37	4.98 \pm 0.58
HB	11.50 \pm 1.38	12.45 \pm 0.93	10.64 \pm 0.94	11.55 \pm 1.30	12.22 \pm 1.28	10.70 \pm 1.22	11.46 \pm 1.46
Hct	36.43 \pm 4.28	39.52 \pm 2.89	33.88 \pm 3.17	36.70 \pm 4.14	38.51 \pm 4.01	33.79 \pm 3.39	36.15 \pm 4.40
MCV	67.95 \pm 6.29	64.44 \pm 4.54	63.31 \pm 4.57	63.88 \pm 4.59	71.47 \pm 5.23	72.59 \pm 4.67	72.03 \pm 4.98
MCH	21.51 \pm 2.38	20.31 \pm 1.72	19.92 \pm 1.61	20.12 \pm 1.67	22.73 \pm 2.18	23.07 \pm 2.14	22.90 \pm 2.16
MCHC	31.61 \pm 1.33	31.53 \pm 1.25	31.48 \pm 1.20	31.50 \pm 1.23	31.78 \pm 1.39	31.67 \pm 1.44	31.73 \pm 1.41
RDW	16.75 \pm 1.83	16.78 \pm 1.39	16.64 \pm 1.38	16.71 \pm 1.39	16.76 \pm 2.15	16.82 \pm 2.21	16.79 \pm 2.18

respectively in males, however, the efficiency values were 39.5% and 99.0% respectively in females. The same trend was seen in other indices and formulas such as Kerman Formula #1, Kerman Formula #2 and Mentzer Formula. Moreover, when the two sexes were combined into a single group the efficiencies of detecting BTM and ID were significantly decreased. For instance, England and Fraser Formula effectively detected 81.6% of the overall ID and 66.2% of the overall BTM cases, while Shine and Lal formula effectively detected 99.8% of BTM and 4.0% of ID overall cases.

The discrimination power of the indices and formulas is presented in three different Tables 4–6 to represent the males, females, and combined data respectively. Tables 4, 5 revealed wide and recognizable differences in the discrimination power of the indices and formulas when applied to males and females data. For illustration and in terms of sensitivity (Fig. 2A) Sirdah, Green king, England and Fraser, and Mentzer formulas respectively showed male vs. female values of 77.6% vs. 96.8%; 71.4% vs. 97.4%; 72.0% vs. 97.5%; 73.5% vs. 93.9%. The same trend was detected for other discrimination power variables emphasizing substantial variations in the behavior of these indices and formulas between males and females. The positive predictive value (PPV) of Ehsani, Srivastava, Ricerca, and Kerman 2 formulas respectively indicated male vs female values of (95.0% vs. 76.0%; 82.5% vs. 54.0%; 96.0% vs. 66.0%; 90.0% vs 77.0%). Concomitantly, for all indices and formulas the calculated AUC by the receiver operative characteristic curve showed larger areas for females data compared to males. For instance, the AUC (Fig. 2B) for females and males respectively generated by Sirdah formula were 0.952 vs 0.887, by Mentzer formula were 0.951 vs. 0.890, by Ehsani formula were 0.950 vs. 0.888, and by Kerman1 formula were 0.935 vs. 0.870.

4. Discussion

Hemogram with microcytic and /or hypochromic RBC is a common feature encountered in almost all subjects of BTM or ID. However, these features are not of any diagnostic values unless other particular tests are performed to confirm the diagnosis. In many countries where both disorders, BTM or ID, are prevalent, the differential diagnosis requires confirmation with additional laboratory

Table 3
The efficiency (% and number between brackets) of the different indices and formulas in terms of percentages of the correctly diagnosed cases for males and females separately and as overall BTM and overall ID.

Index	Males				Females				Overall			
	BTM	ID	p-value	z-test	BTM	ID	p-value	z-test	BTM	ID	p-value	z-test
	Bordbar formula: $(80 \cdot \text{MCV} \times 27 \cdot \text{MCH})$	88.5 (177)	67.0 (134)	0.00		92.0 (184)	71.5 (143)	0.00		90.2 (361)	69.2 (277)	0.00
Ehsani Formula: $(\text{MCV} \cdot 10 \times \text{RBC})$	95.0 (190)	70.0 (140)	0.00		76.0 (152)	95.5 (191)	0.00		85.5 (342)	82.8 (331)	0.289	
England and Fraser formula: $(\text{MCV} \cdot \text{RBC} \cdot 5 \times \text{Hb} \cdot 3.4)$	93.0 (186)	64.0 (128)	0.00		39.5 (79)	99.0 (198)	0.00		66.2 (265)	81.5 (326)	0.00	
Green and King Formula: $(\text{MCV}^2 \times \text{RDW} / \text{Hb} \times 100)$	89.0 (178)	64.5 (129)	0.00		60.5 (121)	95.0 (190)	0.00		74.8 (299)	79.8 (319)	0.091	
Huber-Herklotz Index: $(\text{MCH} \times \text{RDW} \times 0.1 / \text{RBC}) + \text{RDW}$	39.0 (78)	71.0 (142)	0.00		26.0 (52)	88.0 (176)	0.00		32.5 (130)	79.5 (318)	0.00	
Kandhro index #1: $(\text{RBC} / \text{HCT} + 0.5 \times \text{RDW})$	23.5 (47)	52.5 (105)	0.00		25.5 (51)	58.0 (116)	0.00		24.5 (98)	55.2 (221)	0.00	
Kandhro index #2: $(\text{RDW} \times 5) / \text{RBC}$	97.0 (194)	31.0 (62)	0.00		74.5 (149)	64.5 (129)	0.03		85.8 (343)	47.8 (191)	0.00	
Keikhaei index: $\text{Hb} \times \text{RDW} \times 100 / (\text{RBC})^2 \times \text{MGHC}$	90.5 (181)	69.0 (138)	0.00		67.5 (135)	95.0 (190)	0.00		79.0 (316)	82.0 (328)	0.285	
Kerman Formula #1: $\text{MCV} \times \text{MCH} / \text{RBC}$	95.0 (190)	60.0 (120)	0.00		89.0 (178)	84.5 (169)	0.184		92.0 (368)	72.2 (289)	0.00	
Kerman Formula #2: $(\text{MCV} \times \text{MCH} / \text{RBC}) \times 10 / \text{MGHC}$	89.5 (179)	72.5 (145)	0.00		77.0 (154)	93.5 (187)	0.00		83.2 (333)	83.0 (332)	0.928	
Matos-Carvalho Index: $(1.91 \times \text{RBC}) + (0.44 \times \text{MCHC})$	97.5 (195)	33.5 (67)	0.00		54.5 (109)	91.5 (183)	0.00		76.0 (304)	62.5 (250)	0.00	
Mentzer formula: $(\text{MCV} / \text{RBC})$	96.0 (192)	65.5 (131)	0.00		77.5 (155)	95.0 (190)	0.00		86.8 (347)	80.2 (321)	0.013	
RDW index: $(\text{RDW} \times \text{MCV}) / \text{RBC}$	93.5 (187)	53.0 (106)	0.00		82.0 (164)	90.5 (181)	0.014		87.8 (351)	71.8 (287)	0.00	
Riceira index: $(\text{RDW} / \text{RBC})$	96.0 (192)	34.0 (68)	0.00		66.0 (132)	68.5 (137)	0.596		81.0 (324)	51.2 (205)	0.00	
Shine - Lal Formula: $(\text{MCV}^2 \times \text{MCH} / 100)$	99.5 (199)	4.0 (8)	0.00		100.0 (200)	4.0 (8)	0.00		99.8 (399)	4.0 (16)	0.00	
Sirachainan index: $1.5 \times \text{Hb} \cdot 0.05 \text{ MCV}$	84.5 (169)	23.0 (46)	0.00		20.0 (40)	83.5 (167)	0.00		52.2 (209)	53.2 (213)	0.779	
Sirdah Formula: $\text{MCV} \cdot \text{RBC} \cdot 3 \times \text{Hb}$	92.0 (184)	73.5 (147)	0.00		61.0 (122)	98.0 (196)	0.00		76.5 (306)	85.8 (343)	0.001	
Srivastava formula: $(\text{MCV} / \text{RBC})$	82.5 (165)	77.0 (154)	0.171		54.0 (108)	97.5 (195)	0.00		68.2 (273)	87.2 (349)	0.00	
^a Zaghloul index #1: $\text{Hb} + \text{Hct} + \text{RBC}$	69.5 (139)	38.5 (77)	0.00		32.5 (65)	69.0 (138)	0.00		59.2 (237)	49.2 (197)	0.005	
^a Zaghloul index #2: $\text{Hb} + \text{Hct} + \text{RBC} - \text{RDW}$	57.5 (115)	46.0 (92)	0.021		30.0 (60)	68.5 (137)	0.00		53.2 (213)	54.0 (216)	0.834	

^a The numbers of the overall truly diagnosed BTM and ID in Zaghloul formulas are not equal to the sum of the individual values of male or females because there are different cut-off values for males and females.

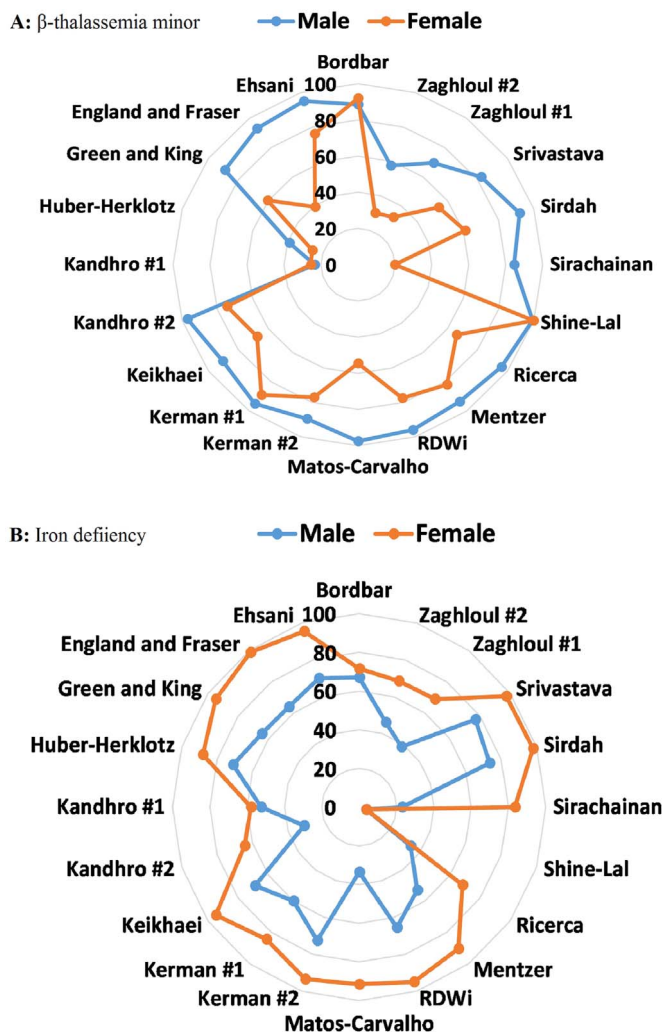


Fig. 1. The efficiency of each index and formula for males and females in correctly diagnosing β -thalassemia minor (A) and iron deficiency (B).

procedures such as quantitation of serum ferritin and HbA₂ levels. The financial burden of these confirmatory tests could be a limiting factor for mass screening programs, especially in developing and poor countries where resources are restricted [10,23]. Consequently, during the last four decades great efforts have been made to evaluate the RBC-based indices or generation of mathematical formulas that could be used to differentiate between blood samples of BTM or ID subjects [6].

Although, the original published indices and formulas revealed very high discrimination power (almost 100%) in terms of the correctly diagnosed cases [3,21], none of the suggested indices or formulas provided an absolute discrimination power when applied thereafter in other settings [10,13,24–30]. The index or formula that offered the highest discrimination power for one population, yet, it showed moderate or low power when applied to other populations.

The widely varied inter-population effectiveness of these indices and formulas could be explained on one or more of different levels. First is the differences of the β -thalassemia mutation spectrum in the different populations. Some prevailing mutations and genetic variants are severe on the phenotype and consequently, on the changes of RBC-indices, while other mutations exert moderate or even mild effects so the changes on RBC-indices are very small [10,31,32]. Second is the difference in the nature of the study samples. In some studies, the evaluations were performed on less homogenous samples to include more males than females or vice versa [19,24,33]. While other studies included blood samples from wide age range, from both sexes [14,24,26,29,33,34]. In some studies, the authors compared thalassemia vs non-thalassemic or other causes of microcytic samples [13], while more serious, is the inclusion of alpha and beta thalassemia as one group [19,22] or including cases with microcytosis /hypochromasia due to chronic diseases (lead poisoning and sideroblastic anemia) in the evaluation studies [25]. Third are substantial variations in the total number of samples, or unbalanced representation of differentiating disorders between the different studies [26,27,34]. Fourth is the inclusion of subjects with late stage of iron deficiency leading to severe anemia [34]. In such studies, patients with lower hemoglobin levels should be excluded to allow a more useful comparison with thalassemia trait, where severe anemia is uncommon feature [35]. Furthermore, evaluation studies should also exclude unusual samples such as hypochromic polycythemia and subjects under iron

Table 4
The discrimination power of the different Mathematical indices and formulas for 400 male data.

Mathematical indices and formulas	Sens (%)	Spec (%)	AUC	+ L.R	- L.R	Youden index (%)	P.P.Value (%)	N.P.Value (%)
Bordbar formula: $(80-MCV \times 27-MCH)$	72.84	85.35	0.830	4.97	0.32	58.19	88.50	67.00
Ehsani Formula: $(MCV-10 \times RBC)$	67.00	93.33	0.888	10.05	0.35	60.33	95.00	70.00
England and Fraser formula: $(MCV-RBC-5 \times Hb-3.4)$	72.09	90.14	0.866	7.31	0.31	62.23	93.00	64.00
Green and King Formula: $(MCV^2 \times RDW/Hb \times 100)$	71.49	85.43	0.860	4.91	0.33	56.92	89.00	64.50
Huber-Herklotz Index: $(MCH \times RDW \times 0.1/RBC) + RDW$	57.35	53.78	0.639	1.24	0.79	11.13	39.00	71.00
Kandhro index #1: $(RBC/HCT + 0.5 \times RDW)$	33.10	40.70	0.555	0.56	1.64	-26.20	23.50	52.50
Kandhro index #2: $(RDW \times 5)/RBC$	58.43	91.20	0.744	6.64	0.46	49.63	97.00	31.00
Keikhaei index: $Hb \times RDW \times 100 / (RBC)^2 \times MCHC$	74.49	87.89	0.872	6.15	0.29	62.38	90.50	69.00
Kerman Formula #1: $MCV \times MCH/RBC$	70.37	92.30	0.870	9.14	0.32	62.67	95.00	60.00
Kerman Formula #2: $(MCV \times MCH/RBC) \times 10/MCHC$	76.50	87.35	0.879	6.05	0.27	63.85	89.50	72.50
Matos-Carvalho Index: $(1.91 \times RBC) + (0.44 \times MCHC)$	59.45	93.06	0.845	8.57	0.44	52.51	97.50	33.50
Mentzer formula: (MCV/RBC)	73.56	94.24	0.890	12.77	0.28	67.80	96.00	65.50
RDW index: $(RDW \times MCV)/RBC$	66.55	89.08	0.872	6.09	0.38	55.63	93.50	53.00
Ricerca index: (RDW/RBC)	59.27	89.47	0.744	5.63	0.46	48.73	96.00	34.00
Shine - Lal Formula: $(MCV^2 \times MCH/100)$	50.90	89.00	0.831	4.63	0.55	39.90	99.50	4.00
Sirachainan index: $1.5xHb-0.05 MCV$	52.32	59.74	0.583	1.3	0.8	12.06	84.50	23.00
Sirdah Formula: $MCV-RBC-3 \times Hb$	77.64	90.18	0.887	7.91	0.25	67.82	61.00	98.00
Srivastava formula: (MCV/RBC)	78.20	81.48	0.871	4.22	0.27	59.68	82.50	77.00
Zaghloul index #1: $Hb + Hct + RBC$	53.05	55.80	0.592	1.2	0.84	8.85	69.50	38.50
Zaghloul index #2: $Hb + Hct + RBC - RDW$	57.60	52.00	0.561	1.2	0.82	9.60	57.50	46.00

Table 5
The discrimination power of the different Mathematical indices and formulas for 400 female data.

Mathematical indices and formulas	Sens (%)	Spec (%)	AUC	+ L.R	- L.R	Youden index (%)	P.P.Value (%)	N.P.Value (%)
Bordbar formula: $(80-MCV \times 27-MCH)$	76.35	89.94	0.895	7.59	0.26	66.29	92.00	71.50
Ehsani Formula: $(MCV-10 \times RBC)$	94.41	79.92	0.95	4.7	0.07	74.33	76.00	95.50
England and Fraser formula: $(MCV-RBC-5 \times Hb-3.4)$	97.53	62.07	0.923	2.57	0.04	59.60	39.50	99.00
Green and King Formula: $(MCV^2 \times RDW/Hb \times 100)$	97.37	70.63	0.928	3.32	0.04	68.00	60.50	95.00
Huber-Herklotz Index: $(MCH \times RDW \times 0.1/RBC) + RDW$	68.42	54.32	0.726	1.5	0.58	22.74	26.00	88.00
Kandhro index #1: $(RBC/HCT + 0.5 \times RDW)$	38.00	43.80	0.519	0.68	1.42	-18.20	25.50	58.00
Kandhro index #2: $(RDW \times 5)/RBC$	67.80	71.70	0.772	2.4	0.45	39.50	74.50	64.50
Keikhaei index: $Hb \times RDW \times 100 / (RBC)^2 \times MCHC$	93.10	74.51	0.932	3.65	0.09	67.61	67.50	95.00
Kerman Formula #1: $MCV \times MCH/RBC$	85.17	88.48	0.935	7.39	0.17	73.65	89.00	84.50
Kerman Formula #2: $(MCV \times MCH/RBC) \times 10/MCHC$	92.22	80.26	0.946	4.67	0.1	72.48	77.00	93.50
Matos-Carvalho Index: $(1.91 \times RBC) + (0.44 \times MCHC)$	93.06	66.80	0.849	2.8	0.1	59.86	54.50	91.50
Mentzer formula: (MCV/RBC)	93.94	80.85	0.951	4.91	0.07	74.79	77.50	95.00
RDW index: $(RDW \times MCV)/RBC$	89.62	83.41	0.936	5.4	0.12	73.03	82.00	90.50
Ricerca index: (RDW/RBC)	67.69	66.83	0.773	2.04	0.48	34.52	66.00	68.50
Shine - Lal Formula: $(MCV^2 \times MCH/100)$	51.02	100.00	0.896	0	0.49	51.02	100.00	4.00
Sirachainan index: $1.5xHb-0.05 MCV$	54.80	51.10	0.553	1.12	0.88	5.90	20.00	83.50
Sirdah Formula: $MCV-RBC-3 \times Hb$	96.82	71.53	0.952	3.40	0.04	68.35	76.50	85.75
Srivastava formula: (MCV/RBC)	95.58	67.94	0.933	2.98	0.07	63.52	54.00	97.50
Zaghloul index #1: $Hb + Hct + RBC$	51.20	50.55	0.53	1.04	0.97	1.75	32.50	69.00
Zaghloul index #2: $Hb + Hct + RBC - RDW$	48.80	49.50	0.523	0.97	1.03	-1.70	30.00	68.50

correction treatment which often give false-positive results [36].

All the above-mentioned points encouraged us to design a study to provide a comparative analysis of the most published indices and formulas based on highly controlled, homogenous, equalized and randomized samples selected from a large pool of eligible records.

The RBC-related indices and parameter, except for RDW and MCHC, were significantly different between the β -thalassemia and iron deficiency groups as well as between males and females of each group. Such differences could be a contributing factor in the varied efficiencies of the published indices and formulas, and explain the variation between the two sexes for the two disorders.

One recognizable observation in our cohort is the inverted efficiency trend of most indices and formulas. The index or formula that provides very high efficiency for β -thalassemia minor in one sex, yet provides very high efficiency for ID in the other sex. Another recognizable observation is the variation in the discrimination power applied to male and female data in terms of the calculated diagnostic indicators: sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and Youden index. The studies of

Table 6
The discrimination power of the different Mathematical indices and formulas for the overall 800 males and females data.

Mathematical indices and formulas	Sens (%)	Spec (%)	AUC	+ L.R	- L.R	Youden index (%)	P.P.Value (%)	N.P.Value (%)
Bordbar formula ($ 80-MCV \times 27-MCH $)	74.59	87.66	0.863	6.04	0.29	62.25	90.25	62.25
Ehsani Formula (MCV-10 × RBC)	83.21	85.10	0.899	5.58	0.2	68.31	85.50	82.75
England and Fraser formula(MCV-RBC-5XHb-3.4)	78.17	70.72	0.825	2.67	0.31	48.89	66.25	81.50
Green and King Formula (MCV ² X RDW/Hb X 100)	78.68	75.95	0.865	3.27	0.28	54.63	74.75	79.75
Huber-Herklotz Index (MCH × RDW × 0.1/RBC) + RDW	58.80	54.10	0.680	1.28	0.76	12.90	32.50	79.50
Kandhro index #1 (RBC/HCT + 0.5 × RDW)	35.40	42.30	0.601	0.61	1.53	-22.30	24.50	55.25
Kandhro index #2 (RDW X 5)/ RBC)	62.14	77.01	0.730	2.7	0.49	39.15	85.75	47.75
Keikhaei_index Hb × RDW × 100/ (RBC)2 × MCHC	81.44	79.61	0.877	3.99	0.23	61.05	79.00	82.00
Kerman Formula #1 MCV X MCH/RBC	76.83	83.00	0.893	4.52	0.28	59.83	92.00	83.00
Kerman Formula #2 (MCV X MCH/RBC) X 10/MCHC	93.04	83.20	0.902	5.54	0.08	66.24	83.25	83.00
Matos-Carvalho Index (1.91 × RBC) + (0.44 × MCHC)	67.00	72.30	0.778	2.42	0.46	39.30	76.00	62.50
Mentzer formula (MCV/RBC)	81.65	85.83	0.895	5.76	0.21	67.48	86.75	80.25
RDW index (RDW × MCV)/RBC	75.65	85.42	0.878	5.19	0.29	61.07	87.75	71.75
Ricerca index (RDW/RBC)	62.43	72.95	0.730	2.31	0.52	35.38	81.00	51.25
Shine - Lal Formula (MCV ² X MCH/100)	50.95	94.12	0.865	8.66	0.52	45.07	99.75	4.00
Sirachainan_index 1.5xHb-0.05 MCV	52.80	52.70	0.559	1.12	0.9	5.50	52.25	53.25
Sirdah Formula MCV-RBC-3 × Hb	84.30	78.49	0.887	3.92	0.2	62.79	76.50	85.75
Srivastava formula (MCV/RBC)	84.26	67.94	0.881	2.63	0.23	52.20	68.25	87.25
Zaghoul index #1 Hb + Hct + RBC	53.90	54.72	0.557	1.19	0.84	8.62	59.25	49.25
Zaghoul index #2 Hb + Hct + RBC - RDW	53.65	53.60	0.547	1.16	0.86	7.25	53.25	54.00

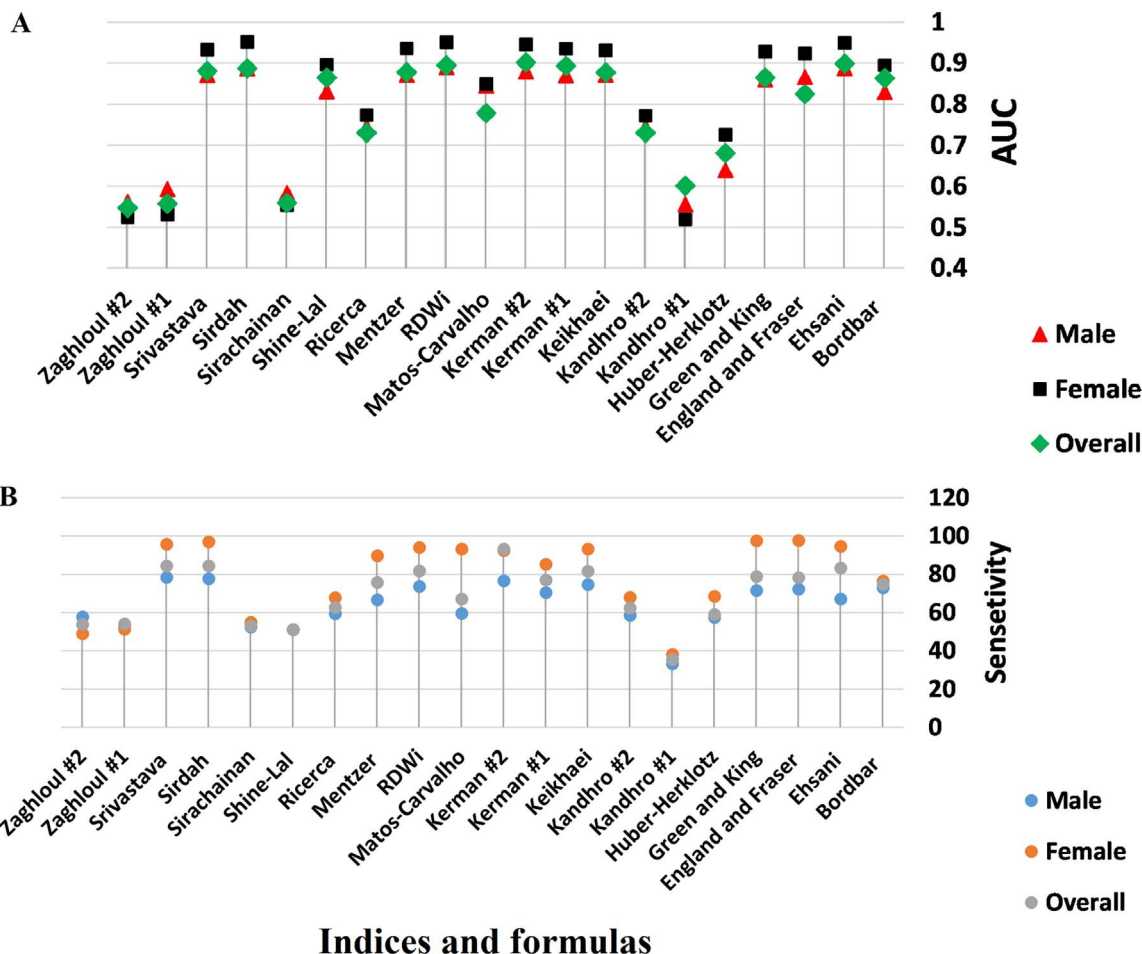


Fig. 2. The discrimination power in terms of: sensitivity (A) and area under the curve [AUC] (B) of the different indices and formulas for males, females and overall.

Batebi et al. and Zaghoul et al. could be among the very few studies that evaluated the discriminating power of some formulas with respect of the sex, where the authors demonstrated valuable sex-based variations in the evaluated discriminating formulas [7,25]. Such variations are not surprising if we realize the sex-based, as well as, to some extent, the age-related differences in the CBC parameters [37–39]. Unfortunately, most of the recently published studies, even our previous article [10], tried to evaluate these functions within the confines of their particular population, tried improve the discrimination power by manipulating the original cut-off and generation of new criteria values or suggested new formulas without any consideration of the sex. In our pilot screening studies we usually discriminate suspicious microcytic and /or hypochromic samples using our previously published formula, Sirdah formula ($MCV-RBC-3 \times Hb$) which when apply to the Palestinian samples from the Gaza Strip provides superior discriminating power compared to most other indices or formulas. However, the current results justify the necessity to revise such approach by considering male and female parameters separately.

Our current study is purposely designed to investigate sex differences in evaluating the reliability of most common indices and formulas in discriminating β -thalassemia minor cases from iron deficiency. The results highlight the importance of considering the sex difference in utilizing these indices and formulas and could direct attention for a new approach in this field, aiming to generate different discriminating indices and formulas for each sex.

5. Conclusion

In conclusion and despite the necessity for performing further laboratory tests to confirm diagnosis, the hematological differences between males and females should be considered when using the mathematical indices and formulas for discriminating between β -thalassemia and iron deficiency. The generation of sex-based indices and formulas could be a suitable approach to minimize variance in the discrimination power observed previously in the discriminating indices and formulas. We also advocate for a multicenter, large-scale study to evaluate the reliability of such indices and formulas with regards to sex and age for application to broader populations.

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Conflict of interest

The Authors declare no conflict of interest related to this work

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