Ahmed *et al.*
The Egyptian Journal of Internal Medicine (2022) 34:60
https://doi.org/10.1186/s43162-022-00147-3

The Egyptian Journal of Internal Medicine

RESEARCH Open Access

Significance of the mathematically calculated red cell indices in patients with qualitative and quantitative hemoglobinopathies

Heba A. Ahmed[1], Safaa A. A. Khaled[2], Eman M. Fahmy[3], Nesreen A. Mohammed[4] and Hamdy F. F. Mahmoud[5,6*]

Abstract

Background: Hemoglobinopathies represent a set of inherited red blood cell (RBCs) disorders, characterized by abnormal hemoglobin molecule. They include qualitative and quantitative hemoglobinopathies, with a structurally abnormal globin chain in the first and defective production in the later. This study assessed, for the first time, the significance of the mathematically calculated RBC indices to identify patients with hemoglobinopathies from normal subjects or iron deficiency anemia (IDA) and to differentiate various types of hemoglobinopathies from each other.

Subjects and methods: The study was a comparative hospital based and included 167 participants with hemoglobinopathies (group 1) and 49 participants with IDA (group 2) as an active comparator. Another 50 healthy volunteers (group 3) were also included. All participants were subjected to medical history, clinical examination, CBC, and HPLC. Next, 10 RBC indices were mathematically calculated from the CBC for each participant.

Results: Gender analysis shows that females represent 36.8% in thalassemia group, 42% in sickle cell disease (SCD) group, and 71.4% in IDA group. The receiver operating characteristic (ROC) curve shows that Ehsani index (EI) is the most reliable screening tool for thalassemics because of showing the highest Youden index and specificity of 41.88% and 88.89%, respectively, followed by Shine and Lal index (SL), with Youden index (YI) value, specificity, and sensitivity equal to 39.78%, 69.70%, and 70.09%, respectively. Similar results were found for IDA. For SCD, SL index is the most suitable screening tool. In conclusion, the mathematically calculated RBC indices are available, cheap, reliable, and sensitive tools for screening patients with hemoglobinopathies.

Keywords: Hemoglobinopathies, Mathematically calculated, Indices

Introduction

Genetic hemoglobin disorders represent the most common gene disorders, with 7% representing carriers in the world population. Hemoglobinopathies denote a set of autosomal recessive diseases which are characterized by the synthesis of structurally abnormal hemoglobin [1]. Qualitative changes due to amino acid substitutions result in hemoglobin variants. Researchers could identify over a thousand hemoglobin variants resulting from changes in the globin chains [2, 3]. Quantitative changes with amino acid insertions, deletions, or mutations in the intervening sequences (introns) match thalassemia and cause lower globin chain production [4]. Thalassemia variant is a type of blood disorder that is caused by a defect in the gene that helps control the production of

*Correspondence: ehamdy@vt.edu

[6] Department of Statistics, Mathematics and Insurance, Faculty of Commerce, Assiut University, Assiut 71515, Egypt
Full list of author information is available at the end of the article

the globin chains protein that makes up the hemoglobin molecule [5]. Some changes (deletions, point mutations, insertions, etc.) in the α-globin genes cause alpha thalassemia. Producing α-globin is measured by the four alleles of HBA1 and HBA2. Regarding the deletional kind, α-thalassemia representing the number of α-globin gene deletions associates with the severity of disease [4]. While a one α-globin gene deletion is found unremarkable (known as the silent carrier), a two α-globin gene deletion (α-thalassemia trait) and three α-globin gene deletion (HbH disease) show various hematological and clinical characteristics. Moreover, a four α-globin gene deletion (Hb Bart's hydrops fetalis) can be found severe and incompatible with life [2].

The more common beta-globin variants include HbS, HbC, HbD, HbE, and HbG. A mutation represents a β-globin subunit findings combined with variant and normal hemoglobin and suggests carrier or trait status, commonly entitled the heterozygote state. Mutations in both β-globin subunits cause disease based on a homozygous or heterozygous expression [5].

Regarding sickle cell anemia (HbSS), mutations are homozygous with producing HbS. Other issues classified under sickle cell disease (SCD), including HbSE, HbSC, and HbSβ-thalassemia, are heterozygous expressions. Irrespective of an α-globin or β-globin variant, the disease severity ranges from insignificant to serious or life threatening [6].

The detection and characterization of a hemoglobinopathy can be done by three tiers workup, which are full blood count, special hematological tests, and DNA testing [7]. Full blood count promotes the success of detection and characterization of the hemoglobinopathy, especially the thalassemia. The key to a thalassemia is a low mean corpuscular volume (MCV) or mean corpuscular hemoglobin (MCH) [8]. Iron deficiency anemia represents the other cause of a low MCV or MCH. However, this result possibly suggests thalassemia in areas with risk ethnic inhabitants [8]. The red cell distribution width (RDW) determines the variation coefficient regarding the MCV. It is higher in iron deficiency not the thalassemia. It may help to point what is more likely [9]. It is worth noting that the MCV may be higher using some conditions. Especially, vitamin B12 and folic acid deficiency cause a higher MCV [10]. One significant hemoglobinopathy is missed if only the MCV or MCH is utilized for the initial screen, namely the HbS carrier. Thus, health professionals or laboratories dealing with populations in which HbS occurs should always include hemoglobin electrophoresis (HbEPG) with the request for a full blood count [11]. Some hemoglobinopathies, mainly HbS, show a normal MCV and normal MCH and may be missed in case of using the full blood count. Since the fetal to adult β-globin switch is not usually complete until about 6 months of life, it is hard to detect β-thalassemia in the neonate based on the full blood count, so special hematological tests and DNA testing will be needed [12].

This study aimed to assess the significance of red cell indices, particularly the mathematically calculated indices to identify patients with hemoglobinopathies from normal healthy controls and IDA which is the most common type of microcytic hypochromic anemia. It also aimed to determine their value in differentiating various types of hemoglobinopathies from each other.

Materials and methods

Study design, settings, and participants

A cross-sectional study was done at the Pediatric and Internal Medicine Departments, Sohag and Assiut University Hospitals, during the period from January 2020 to December 2021. Patients with hemoglobinopathies (group 1) were selected from patients visiting the hematology clinics at the aforementioned departments. Group 1 was further subdivided into patients with quantitative hemoglobinopathies (thalassemia) (group 1A) and patients with qualitative hemoglobinopathies (sickle cell anemia) (group 1B). Forty-nine (49) patients with IDA (group 2) were included as active comparator. Other 50 healthy volunteers of similar age and gender of the patients (group 3) were assembled as controls and were among medical students, as well as medical and paramedical professionals.

Study tools and methods

All groups underwent history aimed to collect demographic data and medical history. Also, all groups were subjected to detailed physical examination, and then, blood samples were taken for complete blood count (CBC). It was done by Cell-Dyn 3700, automated cell counter (Abbott diagnostic, Dallas, USA).

High-performance liquid chromatography (HPLC) was performed for patients only. It was done on D-10 instrument that was manufactured by Bio-Rad Laboratories, USA. Samples were diluted automatically and injected the specimen into the chromatographic station for analysis, based on retention time and proportion of hemoglobin variants. Results were treated using Bio-Rad's clinical data management (CDM) software. HbA2 and F calibrators, two levels of control, were analyzed at the beginning of each run. Normal reference ranges were adopted as in Table S1 (supplementary file) [13].

Red cell indices

Red cell indices were extracted from the automated CBC, for each participant. Another 10 indices were obtained from the CBC after simple mathematical calculations

according to particular equations as detailed in the supplementary file. Those indices included RDW index (RDWI), Mentzer index (or MI), Green and King index (GK), Ehsani index (EI), England and Fraser index (EF), Ricerca index (RI), Srivastava index (SI), Shine and Lal index (SL), Huber-Herklotz index, and Sirdah index (Sir I).

Data analysis

The IBM SPSS (v.20) software for Windows was used to analyze data. Quantitative data were expressed as means ± standard deviation, median, and interquartile range (IQR). In contrast, qualitative data were displayed as number of cases and percentages (%). Shapiro–Wilk test was used to test the normality of the quantitative data. Mann–Whitney *U*-test and Kruskal–Wallis tests were utilized for data that were not normally distributed to detect significant differences between groups. Chi-square (χ^2) test was utilized to compare qualitative variables as appropriate. Fisher-Freeman-Halton exact test was used when χ^2 assumption was not satisfied (there are expected values less than 5). When the rank of the contingency table is high and the χ^2 assumption is not satisfied, the Monte Carlo Freeman-Halton exact test was used. The curve of receiver operating characteristic (ROC) was created for optimum cutoff points of the studied measures in predicting different blood disorders. The area under the ROC curve value (AUC) with 95% confidence interval (CI) was estimated. Optimal cutoff values were defined, namely sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden index (YI). In all statistical tests, 5% significance level was used.

Results

Differences among the study groups as regard demographic and clinical characteristics and transfusion dependency

A total of 266 participants were included in the study they were distributed as in Supplementary Table S2. Table 1 illustrates that there are statistically significant differences between different hemoglobinopathy groups regarding their demographic characteristics, i.e., gender and age of patients (p-value < 0.001 each). Patients with IDA were older in age, and male predominance was commoner in thalassemic patients. Moreover, there is a strong association between clinical presentation and hemoglobinopathy group (Cramer's $V = 0.638$, p-value < 0.05) that means the three groups are different in clinical presentations as the commonest was acute hemolysis in thalassemic patients, while painful crisis was in sickle cell patients. Only pallor was the commonest in IDA patients. Also, Table 1 revealed an association between the patient groups and transfusion type (Cramer's $V = 0.59$, p-value < 0.001), where thalassemic patients are mostly transfusion dependent (55.6%), while 98% SCA and 100% IDA patients are transfusion independent.

Differentiating the study quantitative (β-thalassemia) and qualitative (sickle cell anemia) hemoglobinopathy groups using the mathematically calculated RBCs indices

For differentiating β-thalassemia from other hemoglobinopathy groups, Table 2 shows significant differences in ML-I, EF, SL, EI, and HH (p-value < 0.05). Based on the cutoff values, EI becomes the most consistent index with 88% specificity and 53% sensitivity. ML-I, SL, and HH are discriminators with high sensitivity (53%, 70%, and 59%, respectively) and specificity (81%, 69%, and 63%, sequentially). Other indices are identified by either low specificity or sensitivity causing sequentially an increased number of false-positive or missed β-thalassemia carriers. Based on the ROC curve data, EI continued influential because it scored the best Youden index (41.88) with high sensitivity (53%) and specificity (88%).

For differentiating SCA from other hemoglobinopathy groups, Table 2 shows significant differences in ML-I, SI, SL, and EI (p-value < 0.05). According to the cutoff, SL becomes the most consistent index with 71% of specificity and 76% of sensitivity. ML-I, SI, and EI are discriminators with high sensitivity (26%, 42%, and 50%, respectively) and specificity (94%, 81%, and 74%, respectively). According to the data of the ROC curve data, SL continued influential because it scored the best Youden index (47%) with high sensitivity (53%) and specificity (88%).

Differentiating iron deficiency anemia from the study of other hemoglobinopathy groups using the mathematically calculated RBCs indices

Table 3 shows significant differences in ML-I, GK, EF, EI, and HH (p-value < 0.05). Based on the cutoff values, EI becomes the most consistent index with 56% of specificity and 81% of sensitivity. ML-I, GK, EF, and HH are discriminators with high sensitivity (89%, 97%, 91%, and 93%, respectively) and specificity (46%, 31%, 43%, and 37%, respectively). According to the data of ROC curve, EI continued influential because it scored the best Youden index (37.9%) with high sensitivity (56%) and specificity (81%).

Differentiating various hemoglobinopathy subgroups from the healthy controls using the mathematically calculated RBCs indices

Table 4 shows differentiation of various thalassemia subtypes from healthy controls using the mathematically calculated red cell indices

Using the curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points

Table 1 Differences among the study patients as regard demographic and clinical characteristics and transfusion dependency

Characteristics	Group			*p*-value
	Thalassemia (Group 1A) 117 (54.2%)	Sickle cell anemia (Group 1B) 50 (23.1%)	IDA Group 2 49 (22.7%)	
Age at onset (year)				
Mean ± SD	21.77 ± 13.31	27.3 ± 12.59	33.12 ± 9.35	< 0.001*
Median (IQR)	18 (13–30)	26.5 (20–36.25)	32 (25–39.5)	
Gender				
Male	74 (63.2%)	29 (58%)	14 (28.6%)	< 0.001*
Female	43 (36.8%)	21 (42%)	35 (71.4%)	
Governorate				
Assiut	26 (22.2%)	10 (20%)	3 (6.1%)	
Sohag	67 (57.3%)	31 (62%)	41 (83.7%)	
El Minia	5 (4.3%)	2 (4%)	3 (6.1%)	0.120
Qena	5 (4.3%)	0 (0.0%)	2 (4.1%)	
Aswan	4 (3.4%)	2 (4%)	0 (0.0%)	
Red Sea	5 (4.3%)	3 (6%)	0 (0.0%)	
Luxor	2 (1.7%)	1 (2%)	0 (0.0%)	
New Valley	3 (2.6%)	1 (2%)	0 (0.0%)	
Presentation				
Acute hemolysis	47 (40.2%)	11 (22%)	0 (0.0%)	
Painful crisis	0 (0.0%)	13 (26%)	0 (0.0%)	
Acute abdomen	16 (13.7%)	4 (8%)	2 (4.1%)	
Anemic symptoms	34 (29.1%)	7 (14%)	30 (61.2%)	< 0.0001*
Huge splenomegaly	3 (2.6%)	0 (0.0%)	0 (0.0%)	
Jaundice	15 (12.8%)	2 (4%)	0 (0.0%)	
ACS	0 (0.0%)	2 (4%)	0 (0.0%)	
Obstructive jaundice	2 (1.7%)	11 (22%)	2 (4.1%)	
Chronic bleeding	0 (0.0%)	0 (0.0%)	15 (30.6%)	
Transfusion dependency				< 0.0001*
Dependent	65 (55.6%)	1 (2%)	0 (0.0%)	
Independent	52 (44.4%)	49 (98%)	49 (100%)	

*Statistically significant at 5%. *IDA* iron deficiency anemia, *ACS* acute chest syndrome, *SD* standard deviation, *IQR* interquartile range

in diagnosing thalassemia minor compared to healthy control, Table 4 shows significant differences in SL, RI, EI Sir-I, and HH (p-value < 0.05). Based on the cutoff values, SL becomes the most consistent index with 100% of specificity and 69.5% of sensitivity. RI, EI Sir-I, and HH are discriminators with high sensitivity (69%, 47%, 60%, and 69%, respectively) and specificity (86%, 94%, 88%, and 37%, respectively). According to the data of ROC curve, SL continued influential because it scored the best Youden index (69.5%) with high sensitivity (69.5%) and specificity (100%).

The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing thalassemia intermedia is compared to healthy control. Table 4 shows significant differences in RDWI, SL, RI, EI Sir-I, and HH (p-value < 0.05). Based on the cutoff values, SL is the most reliable index with 100% of specificity and 100% of sensitivity. RDWI, RI, EI Sir-I, and HH are discriminators with high sensitivity (39%, 92%, 60%, 67%, and 82%, respectively) and specificity (100%, 100%, 90%, 86%, and 100, respectively). According to the data of ROC curve, SL continued influential because it scored the best Youden index (100%) with high sensitivity (100%) and specificity (100%).

The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing thalassemia major is compared to healthy control. Table 4 shows significant differences in RDWI, GK, EF, SL, RI, EI Sir-I, and HH (p-value < 0.05). Based on the cutoff values, RI becomes the most consistent index with 90.9% of specificity and 100% of sensitivity. RDWI,

Table 2 The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in differentiating the study hemoglobinopathy groups from each other

Indices	Cutoff	AUC	95% *CI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	*p*-value
Quantitative hemoglobinopathy (*β*-thalassemia)									
RDWI	≤ 241.58	0.533	(0.464, 0.601)	47.86	66.67	62.9	52	14.53	0.405
ML_I	≤ 14.62	0.654	(0.586, 0.717)	53.85	81.82	77.8	60	35.66	< 0.001*
GK	> 73.24	0.561	(0.492, 0.628)	66.67	51.52	61.9	56.7	18.18	0.122
EF	> 14.2	0.586	(0.517, 0.652)	60.68	59.60	64	56.2	20.28	0.029*
SL	≤ 1142.64	0.738	(0.675, 0.796)	70.09	69.70	73.2	66.3	39.78	< 0.001*
SI	≤ 5.25	0.553	(0.484, 0.620)	53.85	62.63	63	53.4	16.47	0.181
RI	> 4.11	0.589	(0.521, 0.656)	51.28	67.68	65.2	54	18.96	0.022*
EI	≤ 20	0.687	(0.621, 0.748)	52.99	88.89	84.9	61.5	41.88	< 0.001*
Sir_I	≤ 28.5	0.549	(0.480, 0.617)	31.62	82.83	68.5	50.6	14.45	0.209
HH	> 25.84	0.606	(0.538, 0.672)	59.83	63.64	66	57.3	23.47	0.007*
Qualitative hemoglobinopathy (sickle cell anemia)									
RDWI	≤ 525	0.5	(0.432–0.569)	72	6.63	18.8	44	21.37	0.994
ML_I	> 28.67	0.605	(0.536–0.670)	26	94.58	59.1	80.9	20.58	0.027*
GK	> 103.6	0.516	(0.447–0.584)	44	81.33	41.5	82.8	25.33	0.783
EF	> 23.7	0.518	(0.449–0.586)	46	84.94	47.9	83.9	30.94	0.757
SL	> 1243.8	0.760	(0.698–0.816)	76	71.08	44.2	90.8	47.08	< 0.001*
SI	> 6.41	0.603	(0.534–0.669)	42	81.93	41.2	82.4	23.93	0.039*
RI	≤ 2.88	0.558	(0.489–0.625)	38	89.76	52.8	82.8	27.76	0.299
EI	> 34.9	0.647	(0.579–0.711)	50	74.10	36.8	83.1	24.1	0.001*
Sir_I	> 43.6	0.586	(0.517–0.652)	44	86.75	50	83.7	30.75	0.111
HH	≤ 22.37	0.528	(0.459–0.596)	44	81.93	42.3	82.9	25.93	0.611

*Statistically significant at 5%

Table 3 The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in differentiating iron deficiency anemia from the study other hemoglobinopathy groups

Indices	Cutoff	AUC	95% *CI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	*p*-value
RDWI	> 241.58	0.547	(0.478, 0.615)	81.63	47.9	31.5	89.9	29.54	0.229
ML_I	> 14.64	0.612	(0.543, 0.677)	89.80	46.11	32.8	93.9	35.9	0.003*
GK	≤ 103.56	0.602	(0.534, 0.668)	97.96	31.74	29.6	98.1	29.7	0.007*
EF	≤ 18.5	0.639	(0.572, 0.703)	91.84	43.11	32.1	94.7	34.95	0.002*
SL	> 901.58	0.574	(0.505, 0.640)	95.92	31.74	29.2	96.4	27.65	0.054
SI	≤ 6.37	0.530	(0.461, 0.598)	95.92	29.94	28.7	96.2	25.86	0.449
RI	≤ 5.18	0.568	(0.499, 0.635)	95.92	28.74	28.3	96	24.66	0.081
EI	> 25	0.616	(0.547, 0.681)	81.63	56.29	35.4	91.3	37.92	0.002*
Sir_I	≤ 43.4	0.517	(0.449, 0.586)	100	26.95	28.7	100	26.95	0.655
HH	≤ 29.81	0.622	(0.553, 0.686)	93.88	37.13	30.5	95.4	31	0.001*

*Statistically significant at 5%

GK, EF, RI, EI, Sir-I, and HH seem also to be discriminator with high sensitivity (43%, 56%, 56%, 93%, 69%, 65%, and 87%, respectively) and specificity (100%, 94%, 88%, 100%, 88%, 68%, and 100%, respectively). According to the data of ROC curve, RI continued influential because it scored the best Youden index (90.9%) with high sensitivity (100%) and specificity (100%).

The curve of receiver operating characteristic of the studied indices for optimum cutoff points in diagnosing sickle cell disease is compared to healthy control.

Table 4 The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing thalassemia subtypes (compared to healthy controls)

Indices	Cutoff	AUC	95% *CI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	*p*-value
Thalassemia minor									
RDWI	> 273.91	0.628	(0.507, 0.738)	34.78	100	100	76.9	34.78	0.125
ML_I	≤ 14.62	0.653	(0.532, 0.760)	56.52	80	54.5	78.4	36.52	0.049
GK	> 81.8	0.540	(0.419, 0.657)	43.48	92	71.4	78	35.48	0.659
EF	≤ 5.6	0.617	(0.496, 0.728)	47.83	84	57.9	77.8	31.83	0.161
SL	≤ 1438.5	0.814	(0.706, 0.895)	69.57	100	100	87.7	69.57	< 0.001*
SI	≤ 4.27	0.641	(0.520, 0.750)	39.13	100	100	78.1	39.13	0.071
RI	> 2.89	0.760	(0.646, 0.853)	69.57	86	69.6	86	55.57	0.001*
EI	≤ 20	0.711	(0.593, 0.811)	47.83	94	78.6	79.7	41.83	0.004*
Sir_I	≤ 33.9	0.693	(0.575, 0.796)	60.87	88	70	83	48.87	0.019*
HH	> 22.38	0.764	(0.650, 0.855)	69.57	98	94.1	87.5	67.57	0.001*
Thalassemia intermedia									
RDWI	> 273.91	0.685	(0.570, 0.786)	39.29	100	100	74.6	39.29	0.009*
ML_I	≤ 14.57	0.562	(0.445, 0.674)	50	82	60.9	74.5	32	0.445
GK	> 88.2	0.511	(0.395, 0.626)	28.57	100	100	71.4	28.57	0.893
EF	> 15	0.585	(0.468, 0.696)	53.57	72	51.7	73.5	25.57	0.235
SL	≤ 1401.7	1	(0.954, 1)	100	100	100	100	100	< 0.001*
SI	> 4.42	0.519	(0.402, 0.633)	75	2	30	12.5	23	0.814
RI	> 3.04	0.974	(0.910, 0.997)	92.86	100	100	96.2	92.86	< 0.001*
EI	≤ 23	0.753	(0.642, 0.844)	60.71	90	77.3	80.4	50.71	0.001*
Sir_I	≤ 34.7	0.804	(0.698, 0.885)	67.86	86	73.1	82.7	53.86	< 0.001*
HH	> 22.52	0.897	(0.807, 0.954)	82.14	100	100	90.9	82.14	< 0.001*
Thalassemia major									
RDWI	> 273.91	0.748	(0.659, 0.824)	43.94	100	100	57.5	43.94	< 0.001*
ML_I	≤ 14.53	0.623	(0.528, 0.711)	53.03	84	81.4	57.5	37.03	0.023*
GK	> 82.12	0.771	(0.683, 0.844)	56.06	94	92.5	61.8	50.06	< 0.001*
EF	> 17.7	0.776	(0.689, 0.848)	56.06	88	86	60.3	44.06	< 0.001*
SL	≤ 1631.2	0.993	(0.956, 1)	93.94	100	100	92.6	93.94	< 0.001*
SI	> 6.24	0.529	(0.434, 0.622)	36.36	94	88.9	52.8	30.36	0.593
RI	> 3.04	0.972	(0.923, 0.994)	90.91	100	100	89.3	90.91	< 0.001*
EI	≤ 24	0.722	(0.631, 0.801)	69.7	88	88.5	68.7	57.7	< 0.001*
Sir_I	≤ 37.2	0.646	(0.551, 0.732)	65.15	68	72.9	59.6	33.15	0.005*
HH	> 22.52	0.935	(0.874, 0.973)	87.88	100	100	86.2	87.88	< 0.001*

*Statistically significant at 5%

Table 5 shows significant differences in RDWI, GK, SI, SL, RI, EF, and HH (p-value < 0.05). Based on the cutoff values, HH becomes the most consistent indices with 92% of specificity and 90.9% of sensitivity. RDWI, GK, EF, SL, SI, and RI seem also to be discriminators with high sensitivity (68%, 75%, 63%, 59%, 63%, and 77%, respectively) and specificity (100%, 100%, 98%, 100%, 96%, and 100%, respectively). According to the data of ROC curve, RI continued influential because it scored the best Youden index (82.9%) with high sensitivity (90.9%) and specificity (92%).

The curve of the receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing sickle cell trait is compared to healthy control. Table 5 shows significant differences in SL, RI, and HH (p-value < 0.05). Based on the cutoff values, SL becomes the most consistent index with 92% of specificity and 78% of sensitivity. RI and HH are discriminators with high sensitivity (46% and 35%, respectively), as well as specificity (100%) for both. According to the data of ROC curve, SLr continued influential because it scored the best Youden index (70.5%) with high sensitivity (78%) and specificity (92%).

Table 5 The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing sickle cell disease and trait (compared to healthy control)

Indices	Cutoff	AUC	95% *CI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	*p*-value
Sickle cell disease									
RDWI	> 273.91	0.807	(0.697, 0.891)	68.18	100	100	87.7	68.18	< 0.001*
ML_I	> 18.98	0.709	(0.590, 0.810)	59.09	90	72.2	83.3	49.09	0.011*
GK	> 88.2	0.798	(0.687, 0.884)	75.29	100	100	89.3	72.73	0.002*
EF	> 24.5	0.780	(0.666, 0.869)	63.64	98	93.3	86	61.64	0.002*
SL	≤ 1639.2	0.815	(0.705, 0.896)	59.09	100	100	84.7	59.09	< 0.001*
SI	> 6.36	0.733	(0.615, 0.830)	63.64	96	87.5	85.7	59.64	0.007*
RI	> 3.04	0.925	(0.839, 0.974)	77.27	100	100	90.9	77.27	< 0.001*
EI	> 45	0.613	(0.491, 0.725)	40.91	94	75	78.3	34.91	0.191
Sir_I	> 44.2	0.646	(0.524, 0.755)	59.09	88	68.4	83	47.09	0.098
HH	> 22.09	0.919	(0.830, 0.970)	90.91	92	83.3	95.8	82.91	< 0.001*
Sickle cell trait									
RDWI	> 273.91	0.549	(0.432, 0.662)	28.57	100	100	71.4	28.57	0.508
ML_I	≤ 19.13	0.517	(0.401, 0.632)	75	6	30.9	30	19	0.824
GK	≤ 56.91	0.617	(0.500, 0.725)	46.43	98	92.9	76.6	44.43	0.164
EF	≤ 0.9	0.6	(0.483, 0.710)	46.43	98	92.9	76.6	44.43	0.232
SL	≤ 1785.6	0.881	(0.788, 0.944)	78.57	92	84.6	88.5	70.57	< 0.001*
SI	> 5.66	0.548	(0.431, 0.661)	50	78	56	73.6	28	0.544
RI	> 3.04	0.770	(0.661, 0.858)	46.43	100	100	76.9	46.43	< 0.001*
EI	≤ 21.1	0.591	(0.474, 0.701)	35.71	94	76.9	72.3	29.71	0.226
Sir_I	≤ 30.9	0.643	(0.527, 0.749)	53.57	94	83.3	78.3	47.57	0.086
HH	> 22.52	0.668	(0.552, 0.770)	35.71	100	100	73.5	35.71	0.016*

*Statistically significant at 5%

Differentiating IDA patients from the healthy controls using the mathematically calculated RBCs indices

The curve of the receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing iron deficiency anemia is compared to healthy control. Table 6 shows significant differences in RDWI, ML-I, SL, RI, sir-I, and HH (p-value < 0.05). Based on the cutoff values, SL becomes the most reliable index with 100% of specificity and 95.9% of sensitivity. RDWI, ML-I, RI, sir-I, and HH seem also to be discriminators with high sensitivity (81%, 65%, 85%, 85%, and 77%, respectively) and specificity (80%, 64%, 100%, 62%, 98%, respectively). According

Table 6 The curve of receiver operating characteristic (ROC) of the studied indices for optimum cutoff points in diagnosing iron deficiency anemia (compared to healthy control)

Indices	Cutoff	AUC	95% *CI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	*p*-value
RDWI	> 240.29	0.857	(0.772, 0.919)	81.63	80	80	81.6	61.63	< 0.001*
ML_I	> 16.73	0.634	(0.531, 0.728)	65.31	64	64	65.3	29.31	0.018*
GK	> 84.74	0.550	(0.447, 0.650)	24.49	96	85.7	56.5	20.49	0.398
EF	≤ 1.4	0.511	(0.409, 0.613)	12.24	98	85.7	53.3	10.24	0.851
SL	≤ 1642.5	0.989	(0.944, 1)	95.92	100	92.2	95.8	95.92	< 0.001*
SI	> 5.66	0.519	(0.417, 0.621)	44.90	78	66.7	59.1	22.90	0.745
RI	> 3.04	0.964	(0.906, 0.991)	85.71	100	100	87.7	85.71	< 0.001*
EI	≤ 36.3	0.592	(0.489, 0.690)	83.67	36	56.2	69.2	19.67	0.107
Sir_I	≤ 38.1	0.787	(0.693, 0.863)	85.71	62	68.9	81.6	47.71	< 0.001*
HH	> 22.38	0.891	(0.812, 0.945)	77.55	98	97.4	81.7	75.55	< 0.001*

*Statistically significant at 5%

to the data of the ROC curve, SL continued influential because it scored the best Youden index (95.9%) with high sensitivity (95.9%) and specificity (100%).

Discussion

Hemoglobinapathies or hemoglobin disorders are a set of blood-associated genetic disorders that may have many causes, effects, and treatments. These genetic diseases affect the shape or number of the red blood cells. They may differ largely from one to another. Some hemoglobinopathies may result in symptoms that threaten life. In contrast, other disorders do not result in any medical issues or even symptoms. Common hemoglobinapathies include sickle cell disease (SCD) and thalassemia (alpha and beta) [14]. According to Tanhehco et al., sickle cell disease and β-thalassemia (BT) are the most popular inherited illnesses, which cause high mortality rates worldwide [15]. The highest rate of affection recorded for both diseases was in the Mediterranean and Middle East area [16].

IDA is often caused by malnutrition. It is a common disease among women, children, and the elderly. It is also associated with many medical conditions and thus complicates the differential diagnosis [17–20]. This study was conducted to create a new approach that could help hematologists and physicians identify a group of thalassemic patients and SCD patients from IDA and normal subjects. This approach is based on both clinical, laboratory, and mathematically calculated RBC indices. Thus, the study included three groups: hemoglobinapathies, IDA, and healthy controls. Patients with hemoglobinopathies were subgrouped according to their diagnosis. All the study participants were subjected to history, clinical examination, and laboratory investigations. Next, mathematical calculation of RBCs indices was done for all participants.

Clinical examination shows that about two-fifth of thalassemic patients suffer acute hemolysis compared to only one-fifth in those with SCD; also, it shows that about nearly two-thirds of IDA patients have anemic manifestation in their blood, which is actually a very high percent compared to only 14% and 29% in SCD and thalassemia patients, respectively. Also, about 31% of IDA patients have chronic bleeding, while it is completely absent in SCD and thalassemia samples. At this case, we assume that physicians can consider the clinical examination as the first stage of distinguishing among thalassemia, SCD, and IDA disorders.

Gender analysis shows that females represent 36.8% in thalassemia group, 42% in SCD group, and 71.4% in IDA group. The result matches the findings of Ghafoor et al., who carried out a study on thalassemic patients and found that female represented about 35.7% [18]. Ceglie et al. studied SCD patients; the percentage of females in their study was 41% [19]. Levi et al. showed female predominance in IDA [20]. These findings confirm that females are more likely to be affected with IDA than SCD and thalassemia. However, these results are not a credible index of drawing differences among SCD, thalassemia, and IDA.

In the current study, we examined the receiver operating characteristic curve according to AUC, 95% CI, cutoff, sensitivity, specificity, positive predictive value, negative predictive value, and Youden index of 10 indices to predict thalassemia, which is often transfusion dependent from other hemoglobinopathy. This result shows that EI index is the most reliable for thalassemic patients because of showing the greatest Youden index and specificity, followed by the SL index. A similar result for IDA, El index could be used to differentiate IDA from other hemoglobinopathies. For SCD, SL index is the most suitable for detecting SCD disorder as it has the highest Youden index and sensitivity. In 2019, Siswandari et al. found that Mentzer index gave the greatest values of reliability to distinguish between β-thalassemia trait and IDA [21]. A similar result was found by Aysel et al. [21, 22].

According to Ferrara et al., RDWI showed the highest sensitivity (78.9%). However, the England and Fraser index showed the highest specificity and Youden's value (99.1 and 64.2%, respectively). The greatest efficiency was shown by the Green and King index (80.2%) in 458 children aged 1.8–7.5 years with mild microcytic anemia [23]. AlFadhli et al. (2007) tested nine indices in participants with microcytic anemia and determined validity by the Youden's index, sensitivity, and specificity. They provided that the EF index showed the greatest value on the Youden's index (98.2%) for properly predicting BTT and IDA [24]. According to our study, the Sir_I and RDW indices showed the lowest values of the Youden's index, i.e., 14.45% and 14.53%. According to Ehsani et al., the best discrimination indices as shown by Youden was the Mentzer index (90.1%) and the Ehsani index (85.5%) [25] which overlap with our results in this study. The reason for the differences of interpretation regarding the effectiveness of different ROC indices in predicting thalassemia from other Hb disorders might be traced back to the mutation spectrum differences in the thalassemia disease among various populations.

Little was found in literature about the value of the mathematically calculated red cell indices in sickle cell disease and trait subjects. This study showed that SL index is the best screening tool for SCD.

Conclusion

EI index is the most reliable screening tool for thalassemia, followed by SL index. Similar results were found for IDA, and El index could be used to predict IDA from other Hb variants. In SCD, SL index is the most suitable index for detecting SCD disorder. Based in these results,

we recommend the use of the mathematically calculated RBCs indices for rapid detection and identification of hemoglobinopathies at the outpatient clinic. The advantage of their inclusion in the diagnostic and screening workup of hemoglobinopathies is that they are widely available, sensitive, and relatively cheap measures

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43162-022-00147-3.

Additional file 1: Table S1. Adult reference ranges of blood indices.
Table S2. Study groups and distribution of hemoglobinapathies among the study patients.

Acknowledgements
The authors wish to thank all patients and controls who contributed in the study.

Authors' contributions
KSAA suggested the research topic, formulated its aims, and put the work plan. KSAA, AHA, and FEM collected data. KSAA and HHA extracted the new indices from patients' blood pictures. MNA and MHFF did the statistical analyses and interpreted results. All authors edited the original draft. MHFF revised the article and did preliminary corrections. All authors revised the manuscript before submission and agreed on the journal. The authors read and approved the final manuscript.

Funding
None

Availability of data and materials
Not applicable

Declarations

Ethics approval and consent to participate
The study complies with the Declaration of Helsinki; moreover, it was approved by the research ethical committee, Sohag Faculty of Medicine. All participant provided a written informed consent before enrollment in the study.

Consent for publication
All the authors approved.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Clinical Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt. [2]Department of Internal Medicine, Clinical Hematology Unit, Assiut University Hospital, Faculty of Medicine / Unit of Bone Marrow Transplantation, South Egypt Cancer Institute, Faculty of Medicine, Assiut University, Assiut, Egypt. [3]Department of Pediatrics, Faculty of Medicine, Sohag University, Sohag, Egypt. [4]Public Health and Community Medicine, Faculty of Medicine, Sohag University, Sohag, Egypt. [5]Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA. [6]Department of Statistics, Mathematics and Insurance, Faculty of Commerce, Assiut University, Assiut 71515, Egypt.

Received: 6 May 2022 Accepted: 18 July 2022
Published online: 04 August 2022

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