Application of Erythrocyte Indices for Screening Thalassemia Trait in Jiujiang Prefecture

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Background: *This study aimed to analyse the application efficiency of 11 erythrocyte indices in Jiujiang Prefecture.*
Methods: *A total of 2379 patients with suspected thalassemia were enrolled in Jiujiang Maternal and Child Health Hospital. Thalassemia genes were detected by reverse dot blot hybridization. A receiver operating characteristic (ROC) curve was used to analyse and calculate the sensitivity, specificity, Youden's index (YI), positive likelihood ratio(+LR) , negative likelihood ratio(-LR) and area under the curve (AUC) of the erythrocyte indices.*
Results: *1) Thalassemia carriers in Jiujiang Prefecture mainly had α- thalassemia and β-thalassemia, with a detection rate of 11.60%. Therefore, --SEA/αα(50.35%) and -α3.7/αα(39.86%%) predominated in α-thalassemia, while the most common genotypes of β-thalassemia were βIVS-II-654/βN, βCD41-42/βN, and βCD17/βN, accounting for 64.29%,15.87% and 9.54%, respectively. 2) Of the 11 erythrocyte indices, Green and King (G&K) exhibited the highest diagnostic efficiency, with an AUC above 90%, high sensitivity and good specificity, followed by England and Fraser (E&F).*
Conclusion: *1) Analysis of thalassemia genotypes can provide a theoretical basis for thalassemia screening, prenatal diagnosis and genetic counselling. 2) Compared with other erythrocyte indices, Green and King (G&K) showed higher application value for thalassemia screening, with the largest AUC above 90%.*

BACKGROUND

Thalassemia, a hereditary haemolytic anaemia caused by the reduction or deletion of globin chain synthesis, possesses a wide distribution in tropical and subtropical regions worldwide and derives its name from its high incidence on the Mediterranean coast and in Southeast Asian countries. Due to population migration, thalassemia has become a significant health issue worldwide.[1] According to statistics, approximately 70,000 neonates are diagnosed with thalassemia annually.[2] Currently, erythrocyte parameters (MCH, MCV) and haemoglobin determination are mainly used at home and abroad to screen children with thalassemia.[3,4] Thalassemia gene detection is considered the gold standard for the diagnosis of thalassemia.[5,6] However, thalassemia gene detection is characterized by its high expenditure, complicated technique and requirements for special instrumentation, making it difficult for mass survey in many medical institutions, especially primary medical establishments. In addition, erythrocyte parameters and haemoglobin determination have a relatively low screening sensitivity and specificity in mild thalassemia, especially patients with silent thalassemia. Due to their unsatisfactory curative effects, these techniques cannot fully meet clinical needs. A multitude of screening formulas using erythrocyte parameters have been established by

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many foreign scholars for convenient and economical screening in thalassemia carriers. However, due to the ethnic characteristics and regional differences in thalassemia genes, the application value of these formulas in other regions needs to be re-evaluated.[7,8] Based on the analysis of sensitivity, specificity, Youden's index (YI) and area under the ROC curve (AUC), we evaluated the applicatin efficiency of 11 erythrocyte indices studied by foreign scholars in Jiujiang Prefecture, Jiangxi Province.

MATERIALS AND METHODS

Subjects

A total of 2379 patients with microcytic anaemia determined by erythrocyte parameters (Hb, MCV <79fl, MCH <27pg) or abnormal haemoglobin electrophoresis to be suspected thalassemia patients from January 2020 to August November 2022 in Jiujiang Maternal and Child Health Hospital were retrospectively enrolled. The age of patients ranged from 0.08 to 60 years old, which included infants, children and adults, but not newborns because the enrolled subjects were limited. Patients were divided into the experimental group and the control group according to the results of thalassemia gene detection and the serum ferritin test, Patients with no blood analysis results and no serum ferritin results were excluded, in addition to thalassemia patients complicated with iron deficiency anaemia. Thalassemia patients were assigned to the experimental group, and iron deficiency anaemia(IDA) patients(serum ferritin <10 μg/L) were assigned to the control group. Pregnant Pregnant women were also analysed as a special population, in which pregnant women with thalassemia were assigned to the experimental group and pregnant women with IDA were assigned to the control group. This study was approved by the medical ethics committee of Jiujiang Maternal and Child Health Hospital(LLSC2021135). Informed consent was not required since this study was retrospective, and data were only collected and tested without other experiments. A waiver for informed consent was waived by the medical ethics committee of Jiujiang Maternal and Child Health Hospital.

Methods

Venous blood was collected on an empty stomach, anticoagulated with EDTA, and run on a BC-6800 automatic haematology analyser (Mindray Company, Shenzhen). Blood cell analytical reagents produced by Mindray Nanjing Biotechnology Co., Ltd.(M-68LD Hemolysis, M-68LB Hemolysis, M-68LH Hemolysis and M-68DR Diluent) were used in this study. Detection indicators included red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit(HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell volume distribution width (RDW-SD) and red blood cell distribution width-coefficient variation (RDW-CV). Serum ferritin was detected by latex agglutinate immunoturbidimetry assay with an Olympus AU5400 analyser(Olympus, Japan). Thalassemia gene detection was based on PCR reverse dot blotting after the extraction of nucleic acid DNA from leukocytes of whole blood on a YN-H16 constant temperature hybridizer(Yaneng Company, Zhuhai, China). Thalassemia gene detection kits were produced by Yaneng Company, and a whole blood DNA extraction kit (centrifugal column type) and Hema9600(Hema Medical Instrument Company, Zhuhai, China) were used in this study. All methods were performed in accordance with the relevant guidelines and regulations. Indoor quality control and interroom quality control were performed in line with the international quality control management standard ISO15189. In addition, erythrocyte parameters, including red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit(HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCH) and red blood cell distribution width (RDW), were resubstituted into the 11 screening and identification formulas for thalassemia studied by foreign scholars (Table 1), and their sensitivity (SEN), specificity (SPE), positive likelihood ratio (+LR), negative likelihood ratio (-LR) and Youden's index (YI) were calculated.

Table 1: Discriminant indices for distinguishing thalassemia trait from healthy subjects in patients

Index	Formula	Cut-off value
England and Fraser (E&F)	MCV – RBC – (5×Hb) – 3.4	≤0
MentzIer(MI)	MCV / RBC	≤13
Srivastava(Sriv)	MCH / RBC	≤3.8
Shine and Lal (S&L)	MCV 2×MCH	≤1.53
Bessman	RDW	≤15
Ricerca(RI)	RDW / RBC	>4.4
Green and King (G&K)	MCV 2×RDW / 100 Hb	≤65
Jayabose (RDW index)	MCV×RDW/RBC	<220
Sirdah(SI)	MCV – RBC – (3×Hb)	≤27.0
RBC count(in million)	RBC	>5.5
Ehsani(EI)	MCV – (10×RBC)	≤17

Microcytic RBC (cut-off values transformed into generally used units: Hb in g/dL; RBC in 10^{12}/L; MCV in fL; MCH in pg; RDW in %).

Statistical analysis

IBM SPSS 22.0 software was used for statistical analysis. The counting data are expressed in percentages (%), and the measurement data are expressed as quartiles. The nonparametric rank sum test (Mann-Whitney U test) and logistic binary regression forwards stepwise regression (Forwards LR) were used to analyse the relevant risk factors. P<0.05 was considered statistically significant. Concurrently, the receiver operating characteristic (ROC) curve was drawm, and analysis of 11 thalassemia screening methods was performed to calculate their sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, Youden's index, and area under the ROC curve (AUC), while the optimal cut-off value was determined according to Youden's index by the software.

RESULTS

Characteristics of the patients

In both the 0.08~3 age group and the 3~14 age group, includinng male and female patients, there was no significant difference in sex between thalassemia patients and IDA patients (p>0.05). However, in the 0.08~3 age group, there were significant differences in ages between thalassemia patients and IDA patients (p<0.05). Because of the lack of eligible patients, in >14 years age group and pregnant women group, only female patients were included, and there was no significant difference between thalassemia patients and IDA patients (p>0.05). All of the patients had no chronic or underlying disease based on a doctor's diagnosis. See Table 2 for details.

Distribution of thalassemia genotypes

Of 2379 patients with suspected thalassemia, 276 cases of thalassemia with genetic abnormalities were detected, with a detection rate of 11.60%(276/2379). There were 143 cases of α-thalassemia (6.01%, 143/2379), 126 cases of β-thalassemia (5.30%, 126/2379) and 7 cases of complex thalassemia (0.29%, 7/2379). A total of 25 thalassemia genotypes were detected. The most common genotypes of α-thalassemia were --SEA/$\alpha\alpha$ (50.35%, 72/143) and -α3.7/$\alpha\alpha$ (39.86%, 57/143), followed genotypes of α-thalassemia were --SEA/$\alpha\alpha$ (50.35%, 72/143) and -α3.7/$\alpha\alpha$ (39.86%, 57/143), followed by -α4.2/$\alpha\alpha$ (3.50%, 5/143), αwsα/$\alpha\alpha$ (1.40%, 2/143), --SEA/-α3.7 (1.40%, 2/143), αCSα/$\alpha\alpha$ (0.70%, 1/143), αQSα/$\alpha\alpha$ (0.70%, 1/143), --SEA/-α4.2 (0.70%, 1/143) and αwsα/--SEA (0.70%, 1/143). The most common genotypes of β-thalassemia were βIVS-II-654/βN, βCD41-42/βN and βCD17/βN, accounting for 64.29% (81/126), 15.87% (20/126) and 9.54% (12/126), respectively,

Table 2: The characteristics of the patients

Group		Sex			Age		chronic or other underlying disease
		male	female	P value	(years)	P value	
0.08<age≤3	Experimental group	10	8	0.157	0.96(0.67~1.88)	0.02	no
	Control group	24	8		0.58(0.42~0.73)		no
3<age≤14	Experimental group	6	4	0.549	6.30(4.75~8.50)	0.368	no
	Control group	3	3		9 7~16		no
age>14	Experimental group	0	120		28 25~31.75	0.093	no
	Control group	0	211		30 27~32		no
Prgnant women	Experimental group	0	105		28 25~31	0.078	no
	Control group	0	174		28.50 25~32		

Table 3 Distribution of thalassemia genotypes in Jiujiang area

Types	Genotypes	Cases (n)	Frequencies (%)	Total (%)	Ratio(n)
α-thalassemia	$--^{SEA}/\alpha\alpha$	72	50.35	143	6.01
	$-\alpha^{3.7}/\alpha\alpha$	57	39.86		
	$-\alpha^{3.7}/-\alpha^{3.7}$	1	0.70		
	$-\alpha^{4.2}/\alpha\alpha$	5	3.5		
	$\alpha^{ws}\alpha/\alpha\alpha$	2	1.4		
	$--^{SEA}/-\alpha^{3.7}$	2	1.4		
	$\alpha^{CS}\alpha/\alpha\alpha$	1	0.70		
	$\alpha^{QS}\alpha/\alpha\alpha$	1	0.70		
	$--^{SEA}/-\alpha^{4.2}$	1	0.70		
	$\alpha^{ws}\alpha/--^{SEA}$	1	0.70		
β-thalassemia	$\beta^{IVS-II-654}/\beta^{N}$	81	64.29	126	5.3
	$\beta^{CD41-42}/\beta^{N}$	20	15.87		
	β^{CD17}/β^{N}	12	9.52		
	β^{-28}/β^{N}	4	3.17		
	$\beta^{CD27/28}/\beta^{N}$	5	3.97		
	β^{E}/β^{N}	2	1.59		
	β^{CAP}/β^{N}	1	0.79		
	β^{-29}/β^{N}	1	0.79		
compound α and β-thalassemia	$\alpha^{QS}\alpha/\alpha\alpha$, $\beta^{CD41-42}$	1		7	0.29
	$-\alpha^{3.7}/\alpha\alpha$, β^{-29}	1			
	$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}$	1			
	$-\alpha^{3.7}/\alpha\alpha$, β^{E}	1			
	$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD41-42}$	1			
	$\alpha^{3.7}/\alpha\alpha$, $\beta^{IVS-II-654}$	1			
	$\alpha^{QS}\alpha/\alpha\alpha$, β^{CD43}/β^{N}	1			
Total				276	11.6

followed by β-28/βN (3.17%, 4/126), βCD27/28/βN (3.97%, 5/126), βE/βN (1.59%, 2/126), βCAP/βN (0.79%, 1/126) and β-29/βN (0.79%, 1/126). The genotypes of the seven cases of complex thalassemia were αQSα/αα, βCD41-42; -α3.7/αα, β-29; -α3.7/αα, βCD41-42; -α3.7/αα, βE and -α4.2/αα, βCD41-42, α3.7/αα, βIVS-II-654, and αQSα/αα, βCD43/βN, as shown in Table 3.

Comparison of erythrocyte parameters between thalassemia patients and IDA patients

In the 0.08~3 age bracket, RBC, HGB, HCT and MCHC of thalassemia patients were significantly higher than those of IDA patients($p<0.05$), while RDW-CV and RDW-SD were significantly lower than those in IDA patients ($p<0.05$). There was no significant difference in MCV or MCH between thalassemia patients and IDA patients ($p>0.05$). In the 3~14 age bracket, RBC, HGB, and HCT of thalassemia patients were significantly higher than those of IDA patients($p<0.05$), while MCV and RDW-SD were significantly lower than those in IDA patients ($p<0.05$). There was no significant difference in MCH, MCHC or RDW-CV between thalassemia patients and IDA patients ($p>0.05$). In patients older than 14 years, there were significant differences in RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD and RDW-CV between thalassemia patients and IDA patients($p<0.05$). RBC HGB, HCT,and MCHC in thalassemia patients were higher than those in IDA patients, while MCV, MCH, RDW-CV and RDW-SD were lower than those in IDA patients. In pregnant women, RBC of thalassemia patients were significantly higher than those of IDA patients ($p<0.05$), while MCV, MCH, RDW-CV and RDW-SD were significantly lower than those in IDA patients ($p<0.05$), and there was no significant difference in HGB, HCT or MCHC between thalassemia patients and IDA patients ($p>0.05$). See Table 4 for details.

Data are presented as median with the interquartile range in parentheses. P values were assessed using the Mann-Whitney U test. *indicates the P value of the comparison between the TT group(0.08<age(years) ≤3) and the IDA group(<0.08age(years) ≤3); and & is the P value of the comparison between the TT group(3<age(years)≤14))and the IDA group(3<age(years) ≤14), and# is the P value of the comparison between the TT group(age(years)>14))and the IDA group(age(years) >14), while $ is the P value of the comparison between the TTgroup(Pregnant women) and the IDA group(Pregnant women).

Table 4: Hematological parameters in the thalassemia trait (TT) and iron deficiency anemia (IDA) groups

Parameters	TT				IDA
	age(years) (0.08~3)	age(years) (3~14)	age(years) (>14)	Pregnant women	age(years) (0.08~3)
RBC(1012/L)	5.43(5.08~5.87)	5.72(4.94~5.86)	4.68(4.75~5.11)	4.71(4.32~5.12)	4.20 3.85~4.60
HGB(g/L)	102(90.50~108.25)	100.00(92.00~106.25)	109 (97.25~119.00)	109 (99.50~119.50)	77.50 65.25~92.00
HCT(%)	32.85(30.65~35.00)	32.65(29.33~35.95)	34.3 (30.88~36.95)	34.5 (31.65~37.00)	26.90 22.45~30.25
MCV( )	60.75(56.58~64.48)	60.50(57.43~61.25)	70.1 (65.48~80.48)	70 (65.35~81.15)	62.90 58.80~67.23
MCH(pg)	18.95(17.78~19.83)	18.30(17.80~18.95)	21.85 (20.73~25.30)	21.9 (20.70~25.55)	18.05 15.98~20.65
MCHC(g/L)	310.50(298.50~316.25)	304.00(295.75~316.25)	320.5 (312.25~325.75)	322 (313.00~326.50)	288.5 (275.25~305.25
RDW-SD	38.80(34.58~42.53)	34.60(33.63~39.15)	38.15(35.50~41.18)	38.2 (35.70~41.35)	41.90 38.80~48.80
RDW-CV(%)	16.25(14.80~18.73)	15.80(15.50~16.68)	15.10(14.10~16.20)	15.1 (14.10~16.20)	18.40 15.88~20.88

Application efficacy evaluation of 11 thalassemia screening formulas reported abroad in Jiujiang Prefecture and comparison with the new thalassemia screening formula constructed by logistics

ROC analysis was performed for the 0.08~3-year age group, 3~14-year age group, >14-year group and pregnant women group, in which Green and King (G&K), Jayabose (RDW index), Ricerca index (RI), England and Fraser (E&F), Sirdah index (SI) and RDW-SD possessed good AUCs for distinguishing TT from IDA, especially Green and King (G&K), with the largest AUCs (94%, 100%, 92% and 92%, respectively), as shown in Table 5a, b, c, and d. The ROC curves of the parameters and the formulas are depicted in Figure1a, b, c, and d.

Table5a: The ROC data elaboration for 0.08~3years group to distinguish TT from IDA

Test Result Variable(s)	Cut-off value	Sensitivity	Speci city	Youden's Index	AUC(95%Cl)	+LR	-LR
England and Fraser (E&F)	7.14	0.94	0.94	0.88	0.95(0.86~1)	14.64	0.06
Bessman	19.25	0.94	0.45	0.4	0.69(0.55~0.84)	1.72	0.12
Sirdah(SI)	29.65	0.94	0.87	0.82	0.92(0.82~1)	7.32	0.06
Ehsani(EI)	13.7	0.83	0.81	0.64	0.85(0.73~0.97)	4.31	0.21
Green and King (G&K)	74.65	0.94	0.94	0.88	0.94(0.87~1)	14.64	0.06
Srivastava(Sriv)	3.85	0.78	0.74	0.52	0.76(0.62~0.89)	3.01	0.3
Jayabose (RDW index)	232.35	0.94	0.9	0.85	0.93(0.86~1)	9.76	0.06
Ricerca(RI)	3.53	0.83	0.87	0.7	0.91(0.83~0.99)	6.46	0.19
Shine and Lal (S&L)	80936.06	0.78	0.45	0.23	0.54(0.37~0.70)	1.42	0.49
MentzIer(MI)	12.68	0.83	0.84	0.67	0.88(0.78~0.98)	5.17	0.2
RBC count(in million)	1.99	0	1	0	0.06(0~0.14)	-	1
logit P1	-166.98	0.89	0.71	0.6	0.82(0.70~0.94)	3.06	0.16
MCH	19.95	0.78	0.35	0.13	0.44(0.28~0.61)	1.21	0.63
MCV	65.4	0.83	0.45	0.28	0.61(0.45~0.78)	1.52	0.37
RDW-SD	38.15	0.5	0.9	0.4	0.73(0.59~0.88)	5.17	0.55
RDW-CV	19.25	0.94	0.45	0.4	0.69(0.55~0.84)	1.72	0.12

Table5b: The ROC data elaboration for 0.08~3years group to distinguish TT from IDA

Test Result Variable(s)	Cut-off value	Sensitivity	Speci city	Youden's Index	AUC(95%Cl)	+LR	-LR
England and Fraser (E&F)	10.69	0.90	1.00	0.90	0.98 0.95~1)	-	0.1
Bessman	17.60	0.90	0.78	0.68	0.78(0.55~1)	4.05	0.128571429
Sirdah(SI)	30.69	0.90	1.00	0.90	0.98(0.95~1)	-	0.1
Ehsani(EI)	16.30	1.00	0.89	0.89	0.97(0.92~1)	9	0
Green and King (G&K)	78.89	1.00	1.00	1.00	1(1~1)	-	0
Srivastava(Sriv)	4.22	1.00	0.67	0.67	0.88(0.74~1)	3	0
Jayabose (RDW index)	223.84	1.00	1.00	1.00	1(1~1)	-	0
Ricerca(RI)	3.78	1.00	1.00	1.00	1(1~1)	-	0
Shine and Lal (S&L)	68256.01	0.70	0.89	0.59	0.83(0.64~1)	6.3	0.3375
MentzIer(MI)	12.07	0.80	1.00	0.80	0.95(0.87~1)	-	0.2
RBC count(in million)	1.24	0.00	1.00	0.00	0.05(0~0.15)	-	1
MCH						-	-
MCV	61.75	0.90	0.89	0.79	0.9 0.75~1	8.1	0.1125
RDW-SD	39.55	0.80	1.00	0.80	0.93 0.82~1	-	0.2
RDW-CV						-	-

Table5c: The ROC data elaboration for adult group(age>14years) to distinguish TT from IDA

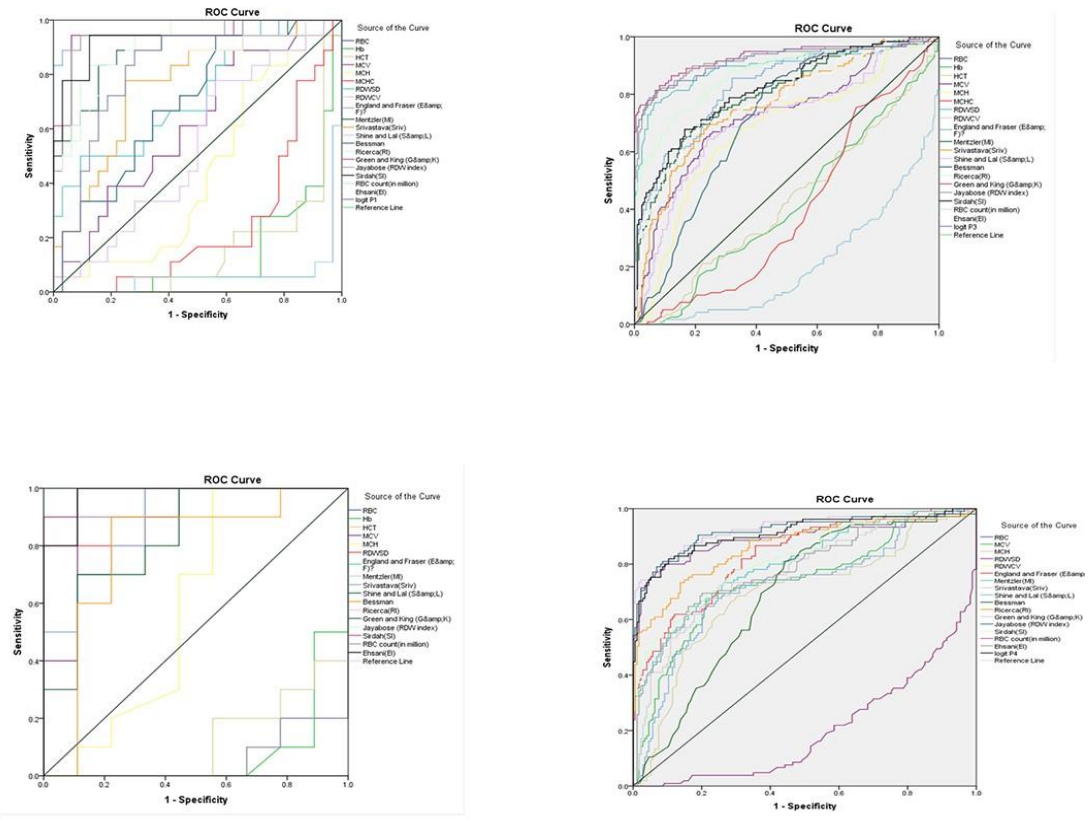
Test Result Variable(s)	Cut-off value	Sensitivity	Speci city	Youden's Index	AUC(95%Cl)	+LR	-LR
England and Fraser (E&F)	17.36	0.85	0.68	0.53	0.83(0.78~0.87)	2.66	0.22
Bessman	16.45	0.83	0.58	0.41	0.71(0.65~0.77)	1.96	0.30
Sirdah(SI)	37.51	0.67	0.83	0.51	0.81(0.76~0.86)	4.07	0.39
Ehsani(EI)	29.55	0.70	0.79	0.49	0.78(0.72~0.83)	3.35	0.38
Green and King (G&K)	86.71	0.83	0.92	0.75	0.92(0.89~0.96)	10.03	0.19
Srivastava(Sriv)	5.18	0.66	0.79	0.45	0.76(0.70~0.81)	3.11	0.43
Jayabose (RDW index)	267.10	0.80	0.93	0.73	0.91(0.88~0.95)	11.80	0.21
Ricerca(RI)	3.43	0.71	0.89	0.60	0.86(0.82~0.91)	6.33	0.33
Shine and Lal (S&L)	120985.51	0.65	0.76	0.40	0.70(0.64~0.76)	2.66	0.47
MentzIer(MI)	16.48	0.67	0.81	0.48	0.79(0.74~0.84)	3.55	0.40
RBC count(in million)	1.85	0.00	1.00	0.00	0.20(0.15~0.25)	-	1.00
logit P3	0.31	0.81	0.93	0.74	0.91(0.87~0.95)	11.13	0.20
MCH	22.95	0.62	0.72	0.34	0.67(0.61~0.73)	2.20	0.53
MCV	72.75	0.65	0.77	0.42	0.72(0.66~0.78)	2.83	0.46
RDW-SD	41.25	0.77	0.93	0.70	0.89(0.85~0.93)	11.29	0.25

Table5d: The ROC data elaboration for pregnant women group to distinguish TT from IDA

Test Result Variable(s)	Cut-off value	Sensitivity	Speci city	Youden's Index	AUC(95%Cl)	+LR	-LR
England and Fraser (E&F)	17.36	0.87	0.64	0.51	0.83(0.78~0.88)	2.43	0.21
Bessman	16.45	0.81	0.56	0.37	0.69(0.62~0.75)	1.83	0.34
Sirdah(SI)	37.51	0.66	0.83	0.48	0.81(0.76~0.87)	3.81	0.41
Ehsani(EI)	29.85	0.70	0.80	0.49	0.78(0.73~0.84)	3.46	0.38
Green and King (G&K)	79.86	0.74	0.98	0.72	0.92(0.88~0.96)	32.31	0.26
Srivastava(Sriv)	5.19	0.65	0.80	0.45	0.77(0.71~0.83)	3.31	0.44
Jayabose (RDW index)	271.39	0.81	0.91	0.72	0.92(0.88~0.95)	9.39	0.21
Ricerca(RI)	3.43	0.73	0.86	0.60	0.86(0.81~0.91)	5.32	0.31
Shine and Lal (S&L)	120985.51	0.65	0.79	0.43	0.71(0.65~0.78)	3.05	0.45
MentzIer(MI)	16.54	0.67	0.80	0.47	0.80(0.74~0.85)	3.41	0.41
RBC count (in million)	2.01	0.00	1.00	0.00	0.19(0.14~0.25)	-	1.00
logit P4	2.54	0.82	0.90	0.72	0.91(0.87~0.95)	7.92	0.20
MCH	23.75	0.69	0.70	0.38	0.69(0.62~0.75)	2.25	0.45
MCV	73.45	0.67	0.79	0.45	0.73(0.67~0.79)	3.14	0.42
RDW-SD	41.65	0.77	0.93	0.70	0.89(0.84~0.93)	11.19	0.25

TT means thalassemia trait; IDA means iron de ciency anemia; AUC, area under the ROC curves; CI, con dence intervals; +LR, positive likelihood ratio; -LR, negative likelihood ratio; HCT, hematocrit; MCV, corpuscular volume; MCH, corpuscular hemoglobin content. logitP1= 40.254-4.239RBC-0.063MCHC,logitP3=8.748-2.342RBC-0.029MCHC+0.262RDW-SD, logit P4=-1.121-2.247RBC+0.264RDW-SD.

Figure 1:



DISCUSSION

Thalassemia is one of the largest monogenic inherited diseases, with 350 million carriers worldwide (accounting for 2% of the total population).[9] Guangxi, Guangdong, Yunnan and Hainan remain high incidence areas of thalassemia in China[10,11] In addition, Jiangxi is adjacent to Guangdong, with a relatively high gene frequency of thalassemia (accounting for 2.23%-9.49%), especially in southern Jiangxi Province (Gannan area), and the gene frequency is as high as 9.44%.[12,13] Our results showed that the gene frequency of thalassemia in Jiujiang Prefecture was 11.60%, higher than that in the Jiangxi census population, but lower than that in Northern Jiangxi Province (Ganbei Area) reported in the study by[14]. The reason for the above phenomenon was because we considered suspected thalassemia patients as the study subjects; therefore, the screening rate was higher than that of the census population but lower than that of suspected thalassemia patients in the haematology department, as reported by.[14] Common types of thalassemia worldwide comprise α-thalassemia and β-thalassemia. Our study showed that the genotypes of α-thalassemia were $--^{SEA}/\alpha\alpha$ (50.35%) and $-\alpha^{3.7}/\alpha\alpha$ (39.86%), while the most common genotypes of β-thalassemia were $\beta^{IVS-II-654}/\beta^{N}$ (64.29%), $\beta^{CD41-42}/\beta^{N}$ (15.87%) and β^{CD17}/β^{N} (9.52%), which was consistent with the studies .[14,15,12] In contrast, the incidence of heterozygote $-\alpha^{4.2}/\alpha\alpha$(3.50%) remained

relatively low in Jiujiang Prefecture. Moreover, our study also demonstrated that, compared with the –$\alpha^{3.7}$ incidence of 22.86% and IVS-II-654 incidence of 40.70% in Jiangxi Province reported by,[16]the the probability of -$\alpha^{3.7}$ (39.86%) and IVS-II-654 (64.29%) heterozygosities remained higher in Jiujiang Prefecture, while thalassemia patients with --^{SEA}/$\alpha\alpha$ (50.35%) and $\beta^{CD41-42}/\beta^{N}$ (15.87%) remained relatively few. Thalassemia is a hereditary chronic haemolytic anaemia caused by the reduction or deletion of globin peptide chain synthesis due to the deletion or mutation of the gene that synthesizes the globin peptide chain. Children with thalassemia, especially moderate and severe thalassemia, suffer from illness and pain throughout their lives. The majority of children with severe α-thalassemia usually die from foetal hypoxia. Even if they survive childbirth, they will die after a few hours due to severe tissue hypoxia, which leads to hydrops fetalis because of mass production of Hb Bart's, increasing the risk of obstetric complications. Children with severe β-thalassemia are asymptomatic at birth and begin to develop progressive anaemia symptoms 3-6 months afterwards. Regular blood transfusion, iron removal treatment or haematopoietic stem cell transplantation are required throughtout their lives for survival. If not treated, they will die before 5 years old. In addition, the treatment requires a high expenditure. According to statistics, one child with severe β-thalassemia needs an annually standardized treatment with an expenditure of approximately 100,000 RMB. In addition, the cost increases with age, which brings substantial mental and economic pressure to the family and society. Therefore, the screening and diagnosis of thalassemia is critical to the prevention of moderate and severe thalassemia. The formula of erythrocyte parameters and erythrocyte indices is considered a thalassemia screening method with simplicity, rapidity and low cost. Currently, a single erythrocyte parameter is reported to be a screening indicator for thalassemia in domestic and foreign literature.[17] In particularly, erythrocyte parameters including MCV <80 fl, and MCH <27 pg, are used as positive criteria for thalassemia screening.[3] Our results showed high sensitivity and specificity of MCV and MCH in the 3~14 years age group, older than14 years age group and pregnant women group, but lower specificity in the 0.08~3 years age group. The AUC and Youden's index of both MCV and MCH were not very good in all of the groups. Only the MCV in the 3~14-year age group exhibited a high application value for thalassemia screening, with an AUC of 0.9 and a Youden's index of 0.79.[18] reported RBC count to be one of the most accurate indicators to distinguish BTT and non-BTT. However, in our study, RBC count was not feasible for thalassemia screening, which was similar to the results of Wickramaratne.[8] Red blood cell distribution width (RDW) is a parameter reflecting the heterogeneity of red blood cell volume, and our study showed that RDW possessed a higher application value for thalassemia screening, especially RDW-SD. Compared with other microcytic anaemia, thalassemia possesses optimal erythrocyte homogeneity, while erythrocytes in IDA vary in size. Meanwhile, our study found that, RBC, HGB, HCT and MCHC in the thalassemia groups were higher than those in the control groups, and the difference was statistically significant ($p<0.05$). Similar to the results of1,[19] the MCV, MCH and RDW in the thalassemia groups were significantly lower than those in the control groups. In addition, we evaluated the application value of 11 erythrocyte index formulas studied by foreign scholars in our study, and the results indicated a maximum application value for Green and King (G&K) in all of the groups, with the largest AUCs of 94%, 100%, 92% and 92%, respectively. England and Fraser (E&F), Sirdah index (SI), Jayabose (RDW index) and Ricerca index (RI) also had good application values. However, Bessman, Ehsani index (EI), Srivastava (Sriv), Shine and Lal (S&L) had lower application value for thalassemia screening. In addition, logitP3 and logitP4 showed good application values in the adult group and pregnant women group, which indicates that the combination of RBCs and RDW is beneficial for the screening of adult thalassemia carriers. A better screening method should have high sensitivity and specificity. High sensitivity screening technology can detect almost all patients, and high specificity can reduce the occurrence of false-positive patients, which will minimize the expenditure of diagnostic testing in the late stage. According to the AUC, our results

showed that Green and King (G&K) had the highest diagnostic efficacy for thalassemia screening in Jiujiang Prefecture. The sensitivity and specificity of Green and King (G&K) were above 90%, which was similar to the reports of[20,21]Moreover, compared with single erythrocyte parameters, such as MCV and MCH, Green and King (G&K) possessed a higher application value for thalassemia screening. Therefore, Green and King (G&K) was considered to be feasible for thalassemia screening in Jiujiang Prefecture. There are still a number of deficiencies in this study. Due to limited conditions, the proportion of infants selected for this study was relatively small, with few cases of thalassemia in this region. Children and adult males enrolled in the study were lacking. The subtype differences in thalassemia were not analysed separately. Therefore, more complete experiments are required to verify the study results in the future. In detail, the proportion of newborn and adult males will be increased, the influencing factors of thalassemia screening will be analysed; and the differences between α-thalassemia, β-thalassemia and their subtypes will be concurrently analysed.

CONCLUSION

Currently, no screening diagnostic method can achieve 100% sensitivity and 100% specificity. The AUC of Green and King (G&K) was higher than those of other erythrocyte index formulas, with a high sensitivity and specificity. Due to the decreased incidence of false-positive patients and reduced expenditure of diagnostic experiments in the late period, Green and King (G&K) is considered feasible for thalassemia screening in Jiujiang Prefecture, Jiangxi Province.

DECLARATIONS

Competing Interests

We state that no con ict of interest for submission of the manuscript and this research received no external funding.

Data availability statement

The data generated and analysed in the presented study are available from the corresponding author on request.

Ethics and informed consent

This study was authorized by the medical ethics committee of Jiujiang Maternal and Child Health Hospital. Informed consent was not required since this study was retrospective, data were only collected and tested without other experiments.

Experimental methods

The complete blood count, serum ferritin and thalassanemia gene test in this study were carried out in strict accordance with the operating procedures. Indoor quality control and inter room quality control were performed in line with the international quality control management standard ISO15189.

REFERENCES

1.Fucharoen S, Weatherall DJ.Progress toward the control and management of thethalassemias. Hematol Oncol Clin North Am 2016;30:359–71.

2.Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010; 115:4331–6.

3.Xu Xiaoqin, Li Xiaoshuang. Clinical significance of combined detection of hematological indexes in screening thalassemia. Our Health 2020;(21):56.

4. Rong P, Gu H, Mao Q, et al. Diagnostic value of HbA2 in different types of thalassemia. Journal of Experimental Hematology 2021;29:217-220.

5.Shi G, Liu Y, Luo Y, et al. Analysis of genotype composition of children with thalassemia in Wuzhou, Guangxi. Journal of Public Health and Preventive Medicine 2021; 32:85-89.

6.Zhong Y, Zhang X, Fan J, et al. Screening of abnormal hemoglobinopathy and β-thalassemia of couples of childbearing age in Northern Guangdong (Yuebei prefecture). Chinese Journal of Family Planning & Gynecotokology 2020; 12:64-66,71.

7.Mudita B, Kumar V, Pandey H et al. Role of Hematological Indices as a Screening Tool of Beta Thalassemia Trait in Eastern Uttar Pradesh: An Institutional Study. Indian J Hematol Blood Transfus 2020; 36:719-724.

8.Wickramaratne K A C, Wijewickrama D C. Screening for beta-thalassemia trait; applicability of red cell indices and parameters - A study in Sri Lanka. Int J Health Sci (Qassim) 2021;15: 29-34.

9.WorldHealthOrganization(WHO).Globalepidemiologyofhemolobindisordersandderivedserviceindicators.Availablefrom:https://www.who.int/bulletinvolumes/86/6/06036673/en/.Accessed11January2020.
10.Zeng YT, Huang SZ. Disorders of hemoglobin in China. J Med Genet 1987;24:578-583.
11.Li B. Evaluation on prevalence and control of thalassemia in Guangdong Province. Journal of Southern Medical University 2015;1-193.
12.Zhong T, Lixia J, Hu X, et al. Epidemiological survey and molecular characterization of thalassemia in urban population of Ganzhou city, China. Chinese Journal of Clinical Laboratory Science 2014;32:697-699.
13.Liu P, Huang J. Analysis of the prenatal screening results of thalassemia in 18704 outpa-tients in south Jiangxi province. Modern Chinese Doctor 2018; 56:16-19,23.
14.Nie Y, Lv X, Lin H, et al. Analysis of the Screening 455 Thalassemia Patients in Jiangxi Region. Experimental and Laboratory Medicine 2017;35:458-460.
15.Liu Chang. Study on the mutation type of thalassemia gene and the cut-off value of hemoglobin A2 in ten provinces of China. Academic Journal of Guangzhou Medical University 2019.
16.Zhuang J, Zhang Na, Wang Y et al. Molecular Characterization Analysis of Thalassemia and Hemoglobinopathy in Quanzhou, Southeast China: A Large-Scale Retrospective Study. Front Genet 2021; 12:727233.
17.Rathod DA, Kaur A, Patel V, et a1. usefulness of cell counter—based parameters and formulas in detection of B—thalassemia trait in areas of high prevalence. Am J clin Pathol 2007;128:585—589.
18.Demir A, Yarali N, Fisgin T, et al. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int 2002;44:612-6.
19.Shen C, Jiang Y-m, Shi H et al. Evaluation of indices in differentiation between iron deficiency anemia and beta-thalassemia trait for Chinese children. J Pediatr Hematol Oncol 2010;32: e218-22.
20.Maskoen AM, Reniarti L, Edhyana S et al. Shine & Lal index as a predictor for early detection of β-thalassemia carriers in a limited resource area in Bandung, Indonesia. BMC Med Genet 2019;20: 136.
21.Lafferty J D, Crowther M A, Ali M A et al. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. Am J Clin Pathol 1996;106: 201-5.