BMJ Paediatrics Open

Reference values for reticulocyte haemoglobin equivalent in healthy Chinese children under 5 years and its associations with various blood parameters

Keith Tsz Suen Tung,[1] Chen Chen,[1] Yung Tuen Chiu,[2] Hung Kwan So ,[1] Chi Chiu So,[2] Patrick Ip [1,3]

To cite: Tung KTS, Chen C, Chiu YT, *et al*. Reference values for reticulocyte haemoglobin equivalent in healthy Chinese children under 5 years and its associations with various blood parameters. *BMJ Paediatrics Open* 2024;**8**:e002736. doi:10.1136/bmjpo-2024-002736

KTST and CC contributed equally.

Received 5 May 2024
Accepted 8 August 2024

[1]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, People's Republic of China
[2]Department of Pathology, Hong Kong Children's Hospital, Hong Kong, People's Republic of China
[3]Department of Paediatrics and Adolescent Medicine, Hong Kong Children's Hospital, Hong Kong, People's Republic of China

Correspondence to
Dr Chi Chiu So; scc131@ha.org.hk

Dr Patrick Ip; patricip@hku.hk

ABSTRACT

Background Reticulocyte haemoglobin equivalent (RET-He) is a useful tool for evaluating recent iron usage irrespective of inflammatory status. This study aims to establish a reference for RET-He among Hong Kong healthy children under the age of 5 years and to investigate the association between RET-He and various blood parameters.

Methods A total of 946 children aged 2–48 months from July 2019 to December 2022 were recruited in this cross-sectional study. The RET-He and other haematological parameters were measured by the haematology analyser from Sysmex XN-9100/XN-1500. The ferritin test was performed with the electrochemiluminescence immunoassay. Interval 2.5th percentile to 97.5th percentile represented the normal RET-He ranges. Linear multiple regression analysis was performed to examine the relation between RET-He and various blood parameters. Receiver-operating characteristic curve analysis revealed the sensitivity and specificity of RET-He in identifying iron deficiency.

Results The RET-He in the study population was approximately normally distributed. The age-specific lower limit of RET-He ranges from 25.81 pg (25–36 months) to 27.15 pg (13–24 months). RET-He was found to be lower in the age group 2–6 months (mean=29.47 pg) and 7–12 months (mean=29.41 pg). Changes in RET-He and haemoglobin in relation to age were observed in both sexes (both $p<0.001$). RET-He was influenced by age, some red blood cell parameters and reticulocyte concentrations (all $p<0.05$). A cut-off value of RET-He ≤27.8 pg was determined for identifying iron deficiency.

Conclusions RET-He levels varied with age, with a relatively lower level in infants than in other age groups. The value below the age-specific lower limit of the reference range of RET-He can be used as a limit for preliminary iron-deficiency screening.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Reticulocyte haemoglobin equivalent (RET-He) is a useful tool for evaluating recent iron usage irrespective of inflammatory status. The reference ranges for RET-He in healthy children have never been established in Hong Kong.

WHAT THIS STUDY ADDS

⇒ This study provides the age-specific reference values for RET-He in healthy Chinese children under 5 years for initial iron-deficiency screening.

⇒ RET-He is significantly associated with other haematological parameters indicating iron-deficiency status, demonstrating a potential predictive tool as an early indicator of iron deficiency in children solely.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ It is feasible to promote iron-deficiency screening with RET-He in young children in Hong Kong since the availability of advanced hematology analyser equipment and the age-specific reference value of RET-He.

⇒ Further research is warranted to evaluate the risk of developing iron-deficiency anaemia when diagnosed with iron deficiency with the established RET-He reference by conducting a prospective study.

INTRODUCTION

Iron-deficiency anaemia (IDA) is a leading global health problem affecting approximately 16.42% of children under 5 years globally.[1 2] Children in this age group are at a critical stage of physical and intellectual development,[3 4] and IDA can negatively impact their mental, physical and social development, leading to poor school performance and work capacity in later years.[5–7] Therefore, early diagnosis and prompt treatment of IDA are critical to prevent the long-term effects of brain iron deficiency (ID).[5 8]

Although bone marrow biopsy is the gold standard for diagnosing ID, it is invasive, painful and carries a risk of bleeding or infection from the puncture site.[8 9] In clinical practice, serum ferritin levels are commonly used to assess iron status.[10] However, diagnosing IDA in patients with reticulocyte haemoglobin equivalent (RET-He) in inflammatory

Table 1 Mean values and reference intervals for various blood parameters (N=946)

Variables	Mean±SD	95% CI	Reference interval $P_{2.5}$–$P_{97.5}$
Erythrocytes (×10^{12}/L)	4.64±0.32	4.62 to 4.66	4.01–5.28
Haemoglobin (g/L)	123.6±7.3	123.2 to 124.1	111.0–138.0
MCV (fL)	78.77±3.37	78.55 to 78.98	72.17–85.40
MCH (pg)	26.72±1.33	26.63 to 26.80	23.80–29.23
MCHC (g/dL)	33.91±0.87	33.86 to 33.97	32.27–35.60
HCT (L/L)	0.37±0.03	0.36 to 0.37	0.30–0.41
RDW (%)	12.48±0.83	12.42 to 12.53	11.30–14.30
Leukocytes (×10^{9}/L)	8.43±2.34	8.28 to 8.58	4.70–13.73
Platelet (×10^{9}/L)	343.04±89.73	337.31 to 348.77	181.00–539.28
MPV (fL)	9.96±0.87	9.91 to 10.02	8.60–11.80
Mentzer index	17.10±1.71	16.99 to 17.20	14.04–20.74
Reticulocytes (×10^{9}/L)	61.88±17.18	60.79 to 62.98	34.57–100.43
RET-He (pg)	30.04±1.54	29.94 to 30.14	26.40–32.90
Ferritin (pmol/L)	149.88±135.27	141.24 to 158.53	31.00–484.15

HCT, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; P, percentile; RDW, red cell distribution width; RET-He, reticulocyte haemoglobin equivalent.

conditions can be challenging as ferritin is an acute phase reactant and its levels are elevated in RET-He in inflammatory conditions.[11 12] Recent studies have shown that RET-He could be a useful tool in evaluating recent iron usage and the state of erythropoiesis.[13] Unlike many iron markers, it is not influenced by inflammation.[11] Therefore, it may be more effective than traditional approaches such as bone marrow iron staining.

The diagnostic usefulness of RET-He has been extensively studied in adults, specifically in cases of functional IDA, iron-restricted erythropoiesis in haemodialysis patients undergoing erythropoietin therapy and in evaluating the response to iron treatment.[12 14] Similarly, in children, RET-He has been thoroughly investigated in early young healthy populations and was found to be a reliable indicator of ID.[15 16] However, there are limited established reference ranges for RET-He in healthy children, especially for Chinese population. One study established reference values of RET-He in healthy children aged 1–11 years of age in Spain but it did not particularly focus on infants, a vulnerable age group of ID.[17] Little is known about how RET-He levels vary with age, gender or erythropoietic activity. Therefore, more research is required to better understand the role of RET-He and its interpretation in different populations.

The study aims to establish a reference for RET-He among Hong Kong healthy children under the age of 5 years and to investigate the association between RET-He and various blood parameters,in particular, the effectiveness of RET-He as a diagnostic indicator for identifying ID in children.

Table 2 Reticulocyte haemoglobin equivalent (RET-He) percentiles according to age groups

	Age group (months)					
RET-He (pg) percentile	2–6 (n=167)	7–12 (n=184)	13–24 (n=289)	25–36 (n=134)	37–48 (n=172)	Total (n=946)
$P_{2.5}$	25.92	26.13	27.15	25.81	27.07	26.40
P_{5}	26.44	26.50	27.70	27.08	27.47	27.24
P_{10}	27.70	27.45	28.70	28.25	28.50	28.07
P_{25}	28.70	28.50	29.80	29.30	29.43	29.20
P_{50}	29.50	29.70	30.70	30.40	30.20	30.20
P_{75}	30.50	30.60	31.60	30.93	30.98	31.00
P_{90}	31.42	31.40	32.50	31.50	31.40	31.90
P_{95}	31.96	32.18	33.10	32.23	31.80	32.40
$P_{97.5}$	32.62	32.64	33.45	32.56	32.00	32.90

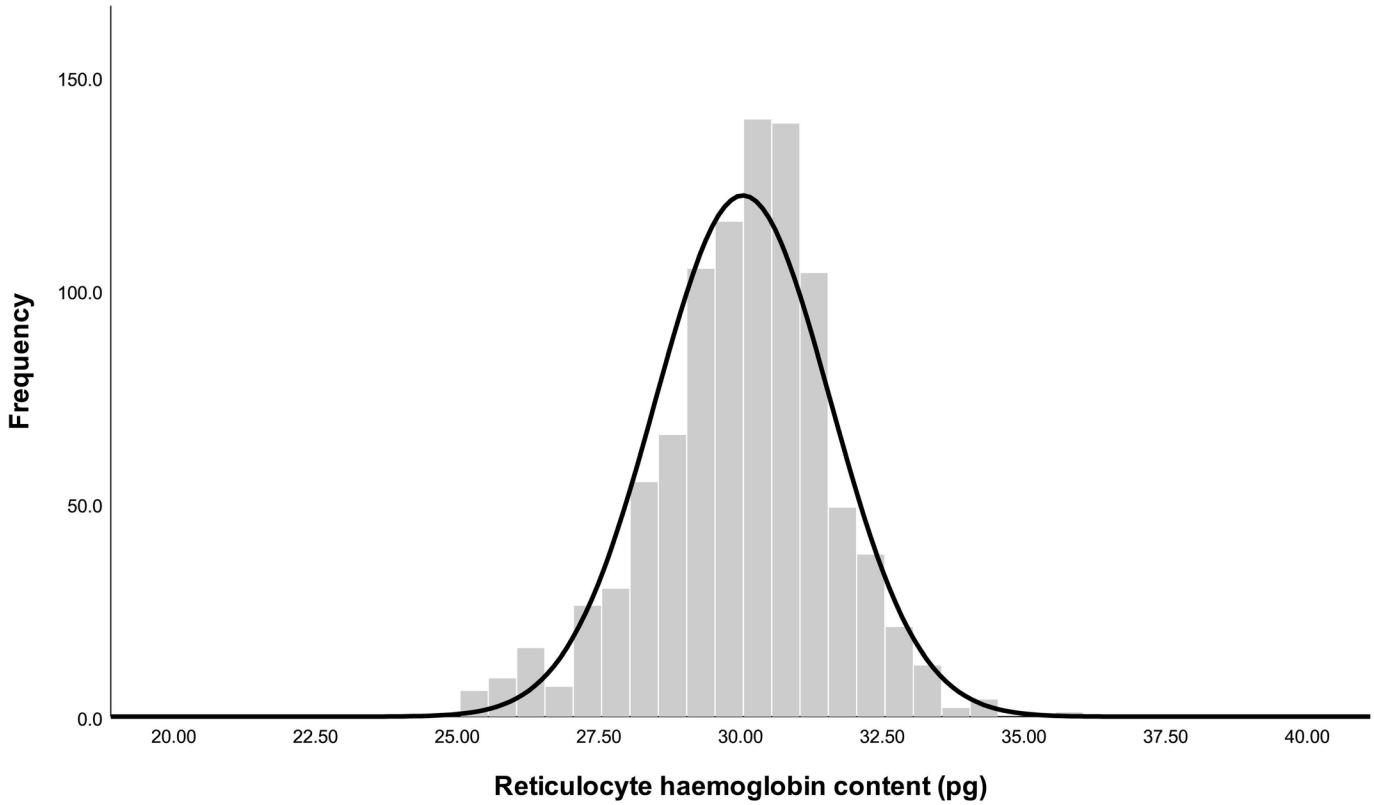

Figure 1 Reticulocyte haemoglobin equivalent (RET-He) distribution among all participants.

METHODS

Study population

This cross-sectional study was conducted from July 2019 to December 2022. 1031 children aged 2–48 months were recruited by stratified random sampling in different districts of Hong Kong, including 6 major maternal and child health centres (MCHCs) and 12 kindergartens. To compile a sampling frame of all kindergartens in Hong Kong, a list of kindergartens from the 'Profile of Kindergarten and Kindergarten-cum-child care Centers Education Bureau' was used. Infants with any major congenital malformations, being born prematurely, or with low birth weight were excluded from this study.

On obtaining informed consent, parents filled out demographic questionnaires and reported the age and sex of their children. Study participants could not have a known haemoglobinopathy, history of anaemia or received a blood transfusion or iron supplement. Peripheral blood samples were collected with anticoagulant tubes by a well-trained phlebotomist.

Laboratory methods

The haematology analyser from Sysmex XN-9100/XN-1500 was used to measure RET-He and other blood parameters. In brief, the reticulocytes are detected in the RET channel of the analyser. The nucleic acids in the immature erythrocytes are stained by the fluorescent dye and measured by the flow cytometry. The haemoglobin concentration is measured in the HGB channel of the analyser by the sodium lauryl sulfate haemoglobin method. The chemical pathology division carried out the ferritin test using an electrochemiluminescence immunoassay on the Cobas E801 immunoassay analyser. The 18 min test involved two incubations. In the first, a sample was combined with specific antibodies to form a complex. In the second, the complex was bound to a solid phase using microparticles. The reaction mixture was then placed in a measuring cell, where the microparticles were magnetically captured and unbound substances were removed. A voltage was applied, causing chemiluminescent emission, which was measured to determine the results. The results were calculated using a calibration curve generated by a 2-point calibration and a master curve provided by the cobas link. According to WHO guidelines 2011, ID is suggested when serum ferritin <27 pmol/L in children up to 5 years old, in the absence of acute/chronic inflammation.

Statistical analysis

Qualitative variables were represented by percentages, while quantitative variables were represented by mean±SD, including 95% CI and reference interval (RI) of 2.5th–97.5th percentile using a parametric approach. RET-He distribution and percentile values were analysed only for healthy children with haemoglobin concentration ≥110 g/L. The laboratory parameters were compared by sex using Student's t-test and by age using one-way analysis of variance. Linear multiple regression analysis was conducted with RET-He as the dependent variable and independent variables including sex, age

Table 3 Comparison of mean values and reference intervals for (A) RET-He and ferritin and (B) reticulocytes and haemoglobin, according to sex, body weight status and age

Variables	N	RET-He (pg)			Ferritin (pmol/L)		
		Mean±SD	$P_{2.5}$ (95% CI)	$P_{97.5}$ (95% CI)	Mean±SD	$P_{2.5}$ (95% CI)	$P_{97.5}$ (95% CI)
(A)							
Sex							
Male	497	30.01±1.52	26.44 (25.74 to 26.88)	32.76 (32.52 to 33.06)	135.38±98.36	31.00 (22.00 to 33.00)	387.85 (308.36 to 440.70)
Female	449	30.08±1.58	26.34 (25.58 to 26.68)	33.18 (33.06 to 33.68)	165.91±165.47	31.00 (22.00 to 33.00)	598.00 (419.00 to 732.35)
P value		0.486			<0.001		
Overweight							
Yes	123	29.87±1.55	26.31 (25.01 to 26.51)	32.88 (32.55 to 33.65)	155.18±111.66	31.25 (13.35 to 33.50)	473.35 (366.70 to 600.90)
No	801	30.07±1.55	26.50 (26.00 to 27.00)	32.90 (32.60 to 33.20)	147.97±136.35	31.00 (26.00 to 33.00)	465.00 (417.01 to 529.75)
P value		0.186			0.577		
Age (months)							
2–6	167	29.55±1.53	26.02 (25.43 to 26.49)	32.29 (31.67 to 32.72)	250.35±251.78	31.90 (11.80 to 36.60)	943.50 (798.40 to 1244.90)
7–12	184	29.56±1.57	26.20 (25.40 to 26.79)	32.49 (32.13 to 32.87)	114.50±71.76	27.20 (23.40 to 33.30)	311.15 (270.20 to 400.30)
13–24	289	30.63±1.55	27.30 (26.88 to 28.10)	33.30 (32.40 to 33.52)	109.34±57.86	31.00 (26.00 to 39.00)	248.80 (191.60 to 282.20)
25–36	134	30.02±1.45	26.03 (24.60 to 26.57)	32.47 (32.24 to 33.24)	151.00±84.34	57.63 (43.25 to 66.25)	346.50 (138.00 to 432.98)
37–48	172	30.08±1.23	27.23 (26.75 to 27.80)	31.97 (31.08 to 32.25)	158.51±96.49	50.13 (35.25 to 58.68)	349.88 (227.78 to 411.14)
P value		<0.001			<0.001		
(B)							
Variables		**Reticulocytes (×109/L)**			**Haemoglobin (g/L)**		
	N	**Mean±SD**	**$P_{2.5}$ (95% CI)**	**$P_{97.5}$ (95% CI)**	**Mean±SD**	**$P_{2.5}$ (95% CI)**	**$P_{97.5}$ (95% CI)**
Sex							
Male	497	59.14±16.58	33.12 (30.66 to 36.52)	95.10 (86.40 to 100.20)	123.9±7.4	111.0 (110.0 to 112.0)	138.0 (135.0 to 139.4)
Female	449	64.92±17.35	34.98 (30.24 to 36.16)	102.38 (92.16 to 108.82)	123.3±0.73	111.0 (110.0 to 112.0)	137.0 (136.0 to 138.0)
P value		<0.001			0.196		
Overweight							
Yes	123	62.61±15.99	35.22 (32.86 to 41.63)	91.43 (82.56 to 98.30)	125.0±7.9	111.1 (109.1 to 112.1)	139.0 (135.9 to 141.0)
No	801	61.61±17.26	34.80 (33.90 to 37.60)	100.10 (94.40 to 105.70)	123.4±7.2	111.0 (111.0 to 112.0)	137.0 (136.0 to 138.0)
P value		0.549			0.018		
Age (months)							
2–6	167	58.50±15.19	34.82 (32.83 to 38.41)	87.82 (75.02 to 92.63)	119.7±6.5	110.0 (109.0 to 110.0)	133.9 (130.9 to 135.9)
7–12	184	62.03±16.83	39.22 (36.27 to 43.11)	99.05 (71.90 to 112.80)	121.9±7.0	111.0 (110.4 to 112.0)	135.0 (133.0 to 136.0)

Continued

Table 3 Continued

Variables	N	RET-He (pg)			Ferritin (pmol/L)		
		Mean±SD	$P_{2.5}$ (95% CI)	$P_{97.5}$ (95% CI)	Mean±SD	$P_{2.5}$ (95% CI)	$P_{97.5}$ (95% CI)
13–24	289	61.96±15.75	35.74 (31.68 to 38.26)	95.62 (91.68 to 100.46)	124.0±6.8	112.0 (111.0 to 113.8)	137.0 (134.2 to 138.0)
25–36	134	64.17±19.85	27.46 (18.58 to 30.62)	102.62 (75.04 to 113.22)	126.2±7.3	113.3 (110.7 to 116.7)	140.0 (134.1 to 143.1)
37–48	172	63.11±19.05	32.25 (27.40 to 33.69)	107.61 (97.76 to 122.94)	126.7±7.0	115.0 (114.5 to 116.0)	139.0 (129.8 to 141.0)
P value		0.045			<0.001		

and BMI z-score. The regression models were adjusted for the sex and age of the child and introduced individually. A 5% level of significance was considered statistically significant. The sensitivity and specificity of RET-He as a marker were determined by receiver-operating characteristic (ROC) plots by MedCalc for window, V.22.001, 95% CI was computed for 2.5 and 97.5 percentiles for 2000 bootstrap resamples using the DescTools Package for R V.4.3.2. Other statistical analyses were conducted by SPSS V.26.0.

RESULTS

A total of 946 children (497 boys and 449 girls) were analysed. Participants were stratified into 5 age groups with a roughly similar distribution, with 167 (17.7%) aged 2–6 months, 184 (19.5%) aged 7–12 months, 289 (30.5%) aged 13– 24 months, 134 (14.2%) aged 25–36 months and 172 (18.2%) aged 37– 48 months.

Table 1 displays the RIs and mean values for various blood parameters while table 2 shows RET-He percentiles for different age groups. Figure 1 shows RET-He was normally distributed in the study population. Table 3 displays the references categorised by age, sex and body weight status. Girls had higher serum ferritin (p<0.001) and reticulocyte (p<0.001) compared with boys, while RET-He and haemoglobin values were similar in both sexes. Haemoglobin level was higher in overweight children than in non-overweight children (p=0.018). Haemoglobin concentration was found to be higher among overweight children. Blood parameters varied with age. RET-He was found to be lower in the age group 2–6 months (mean=26.02) and 7–12 months (mean=26.20).

Table 4 shows the variations in blood parameters (RET-He, serum ferritin, reticulocytes and haemoglobin) by sex and age. Both sexes showed similar age-related variations in haemoglobin and RET-He while young children (ages 2–6 months) of both sexes had greater serum ferritin levels. The mean values of the RET-He and haemoglobin in each age group were similar in both sexes. Girls had higher reticulocyte levels than boys in general. A series of linear regression models were conducted to determine which independent variables were associated with RET-He. As shown in table 5, age-adjusted and sex-adjusted models showed that RET-He was influenced by age, red cell counts (RCCs) (haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCHC, red cell distribution width) and reticulocytes (all p<0.05). There was no significant association between RET-He and sex, body weight status, HCT, leucocytes and ferritin.

RET-He was assessed as a diagnostic marker for ID when the ferritin level was below 27 pmol/L, using ROC analysis (figure 2). Children exhibiting potential thalassaemia symptoms, characterised by MCV below the reference interval (RI) and RCC above RI, were not considered part of the normal population. In the iron-deficient group, the area under the curve was found to be

Table 4 Sex-specific mean values and reference intervals for RET-He, ferritin, reticulocytes, and haemoglobin by age group

Variables	N	Mean±SD	$P_{2.5}$ (95% CI)	$P_{97.5}$ (95% CI)	P value
(A) Males (N=497)					
RET-He (pg)					<0.001
2–6	84	29.43±1.52	26.41 (25.22 to 27.48)	32.66 (32.43 to 33.73)	
7–12	96	29.57±1.64	26.28 (25.41 to 26.98)	32.53 (31.96 to 32.95)	
13–24	156	30.49±1.47	27.30 (26.68 to 28.30)	33.10 (32.20 to 33.64)	
25–36	74	30.22±1.41	25.95 (23.50 to 26.40)	32.52 (32.14 to 33.50)	
37–48	87	30.00±1.21	27.22 (26.03 to 27.77)	31.97 (31.94 to 32.26)	
Ferritin (pmol/L)					<0.001
2–6	84	187.00±156.01	40.15 (26.10 to 53.30)	500.35 (71.10 to 571.50)	
7–12	96	107.03±69.06	29.00 (18.02 to 36.25)	312.55 (293.25 to 419.00)	
13–24	156	108.76±52.68	30.75 (19.13 to 39.50)	238.00 (170.00 to 282.82)	
25–36	74	139.08±62.54	62.65 (53.30 to 71.30)	308.00 (263.00 to 372.65)	
37–48	87	161.98±115.79	49.63 (33.63 to 61.00)	441.13 (30.25 to 605.88)	
Reticulocytes ($\times 10^9$/L)					0.023
2–6	84	54.52±15.31	33.69 (30.68 to 38.43)	87.93 (67.23 to 102.96)	
7–12	96	60.08±17.25	37.35 (31.30 to 48.70)	90.35 (29.31 to 103.10)	
13–24	156	58.88±15.01	35.50 (31.90 to 38.70)	91.25 (82.20 to 99.93)	
25–36	74	59.31±15.82	26.65 (12.93 to 28.99)	92.83 (84.66 to 104.88)	
37–48	87	62.89±19.36	32.42 (24.45 to 34.15)	112.89 (108.03 to 134.78)	
Haemoglobin (g/L)					<0.001
2–6	84	119.5±6.3	110.0 (108.9 to 110.0)	133.0 (130.2 to 136.2)	
7–12	96	122.6±7.2	111.0 (109.0 to 111.6)	137.0 (134.5 to 141.0)	
13–24	156	124.6±7.0	111.0 (109.0 to 112.0)	139.1 (136.3 to 142.3)	
25–36	74	126.7±7.5	113.8 (110.7 to 117.7)	141.9 (137.8 to 146.8)	
37–48	87	126.1±6.9	114.2 (112.3 to 114.3)	138.9 (130.2 to 141.7)	
(B) Females (N=449)					
Variables	**N**	**Mean±SD**	**$P_{2.5}$ (95% CI)**	**$P_{97.5}$ (95% CI)**	**P value**
RET-He (pg)					<0.001
2–6	83	29.66±1.54	26.01 (24.49 to 26.49)	32.10 (29.80 to 32.70)	
7–12	88	29.54±1.49	26.25 (25.11 to 27.08)	32.05 (31.31 to 32.73)	
13–24	133	30.78±1.63	27.19 (26.15 to 27.88)	33.71 (32.92 to 34.25)	
25–36	60	29.78±1.49	26.58 (25.16 to 27.75)	32.35 (32.01 to 33.21)	
37–48	85	30.16±1.24	27.31 (26.57 to 28.27)	31.90 (30.51 to 32.51)	
Ferritin (pmol/L)					<0.001
2–6	83	314.46±308.94	31.45 (1.13 to 33.85)	1038.88 (105.28 to 1324.75)	
7–12	88	122.57±74.12	25.70 (15.40 to 32.70)	300.30 (203.68 to 380.60)	
13–24	133	110.02±63.60	31.90 (23.80 to 45.80)	258.30 (107.60 to 315.00)	
25–36	60	165.70±103.91	52.33 (22.80 to 61.65)	470.45 (284.90 to 698.68)	
37–48	85	154.96±72.12	54.83 (29.83 to 62.65)	340.25 (224.43 to 417.50)	
Reticulocytes ($\times 10^9$/L)					0.086
2–6	83	62.52±14.05	35.79 (29.07 to 37.44)	86.10 (71.53 to 89.60)	
7–12	88	64.17±16.20	39.82 (35.97 to 40.59)	100.77 (76.09 to 116.24)	
13–24	133	65.58±15.89	40.07 (35.35 to 45.24)	96.18 (84.16 to 101.26)	
25–36	60	70.16±22.64	32.49 (22.37 to 47.17)	129.25 (126.80 to 162.91)	
37–48	85	63.33±18.83	32.99 (24.69 to 48.51)	103.79 (81.98 to 120.65)	

Continued

Table 4 Continued

Variables	N	Mean±SD	$P_{2.5}$ (95% CI)	$P_{97.5}$ (95% CI)	P value
Haemoglobin (g/L)					<0.001
2–6	83	119.8±6.6	111.0 (110.0 to 112.0)	134.0 (130.9 to 138.0)	
7–12	88	121.1±6.8	111.0 (109.8 to 112.0)	134.0 (133.2 to 136.0)	
13–24	133	123.4±6.6	113.0 (112.0 to 115.0)	136.7 (134.4 to 139.3)	
25–36	60	125.5±7.0	112.4 (107.8 to 114.8)	138.0 (133.0 to 140.0)	
37–48	85	127.3±7.2	115.1 (112.1 to 119.7)	140.6 (128.0 to 144.4)	

Males (N=497) Females (N=449).
RET-He, reticulocyte haemoglobin equivalent.

0.842 with p<0.0001. A cut-off value of RET-He ≤27.8 pg was determined, which showed a sensitivity of 70.8% and specificity of 90.9% for identifying ID. Additionally, the RET-He of the children with suspected thalassaemia that was excluded from the ROC analysis was further studied. It was observed that out of 82 suspected thalassaemia cases, 80 had an RET-He value ≤27.8 pg.

DISCUSSION

This study established reference values for RET-He in healthy children under 5 years old in Hong Kong with the reference interval of $P_{2.5}$–$P_{97.5}$. RET-He levels varied with age, with lower levels in infants than other age groups. An age-specific value below the lower limit of the reference range of RET-He can be used as a limit to determine ID. There were no significant gender differences in RET-He levels. RET-He was influenced by factors such as age, haemoglobin levels and reticulocyte levels, among others. These findings contribute to a better understanding of RET-He and its interpretation in the context of iron status in children.

Table 5 The estimated effect of associated factors on RET-He

	Crude			Adjusted*		
Variables	β (95% CI)	R^2	P value	β (95% CI)	R^2	P value
Sex						
Male	−0.03 (−0.09 to 0.04)	0.001	0.486	−0.02 (−0.09 to 0.04)	0.010	0.492
Female	1.00		–	1.00		–
Overweight						
Yes	−0.04 (−0.11 to 0.02)	0.002	0.186	−0.04 (−0.10 to 0.03)	0.012	0.270
No	1.00		–	1.00		–
Age	0.10 (0.03 to 0.16)	0.009	0.003	0.10 (0.03 to 0.16)	0.010	0.003
Haemoglobin (g/L)	0.20 (0.14 to 0.27)	0.042	<0.001	0.19 (0.13 to 0.26)	0.043	<0.001
MCV (fL)	0.53 (0.47 to 0.58)	0.280	<0.001	0.58 (0.52 to 0.63)	0.294	<0.001
MCH (pg)	0.63 (0.58 to 0.68)	0.400	<0.001	0.65 (0.60 to 0.70)	0.404	<0.001
MCHC (g/dL)	0.36 (0.30 to 0.42)	0.129	<0.001	0.38 (0.32 to 0.44)	0.152	<0.001
HCT (L/L)	0.00 (−0.06 to 0.06)	0.000	0.987	−0.03 (−0.10 to 0.04)	0.011	0.361
RDW (%)	−0.33 (−0.39 to to 0.27)	0.110	<0.001	−0.35 (−0.41 to to 0.29)	0.127	<0.001
Leukocytes ($\times 10^9$/L)	−0.06 (−0.13 to 0.00)	0.004	0.050	−0.04 (−0.11 to 0.03)	0.011	0.255
Platelet ($\times 10^9$/L)	−0.11 (−0.17 to to 0.05)	0.012	<0.001	−0.10 (−0.16 to to 0.03)	0.019	0.003
MPV (fL)	−0.07 (−0.13 to 0.00)	0.004	0.042	−0.06 (−0.12 to 0.01)	0.013	0.080
Mentzer Index	0.42 (0.36 to 0.47)	0.174	<0.001	0.42 (0.36 to 0.48)	0.182	<0.001
Reticulocytes ($\times 10^9$/L)	0.18 (0.12 to 0.25)	0.033	<0.001	0.18 (0.11 to 0.24)	0.040	<0.001
Ferritin (pmol/L)	−0.01 (−0.07 to 0.05)	0.0001	0.751	−0.01 (−0.07 to 0.06)	0.009	0.873

*Adjusted for sex and age of the child.
HCT, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RDW, red cell distribution width; RET-He, reticulocyte haemoglobin equivalent.

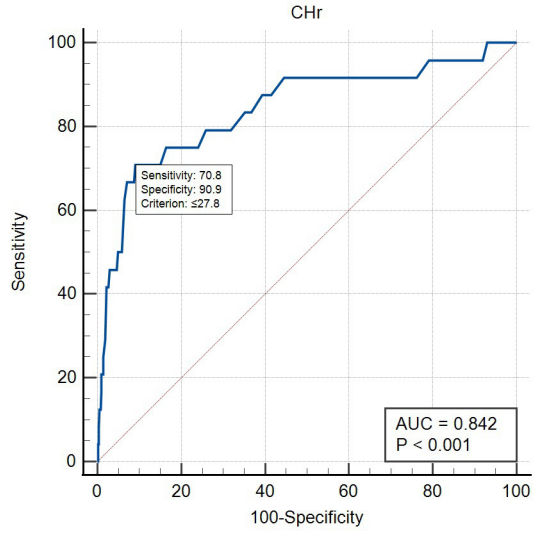

Figure 2 Receiver-operating characteristic (ROC) analysis of reticulocyte haemoglobin equivalent (RET-He) in the diagnosis of iron deficiency.

In this study, the mean RET-He level is 30.04 pg in children aged 2–48 months, which is slightly below previously reported data with broad age ranges 30.5 pg (6 months to 5 years),[18] 30.8 pg (15 days to 19 years),[19] 30.9 pg (1–11 years).[17] As the study has a higher composition of infants, who are more vulnerable to ID than older children,[20] the population was further categorised into five subgroups, and RET-He reference values were detailed in each group. Based on the $P_{2.5}$ level of RET-He, RET-He lower limit reference values were proposed among age groups. These values ranged from 25.92 pg to 27.15 pg in different age groups. Previously reported lower limit reference values for ID in healthy young children range from 24.9 pg to 27.5 pg, including 25.6 and 24.9 pg (4–12 months),[21] 25.6 pg (1–2 years),[17] 27.3 pg (3–5 years),[17] 27.5 pg (9–12 months, 6 months–5 years).[16 18] Given the reference variations in different age groups, it is necessary to apply a local age-specific reference in clinical medicine when initially assessing ID status with complete blood count (CBC).

The mean RET-He fluctuated with age with a lower level in infants than children aged over 12 months old. Iron from mothers provides necessary iron for growth at birth but iron storage is depleted around 4–6 months since dietary iron becomes the sole source of iron required for continued growth.[22] Breast milk, vegetables, fruits and formula milk tend to be low in iron.[22] Infants have increased iron demand to support their rapid growth at this stage, making them more vulnerable to ID.[23] No significant gender difference was found in RET-He, aligning with previous studies.[17 18 24] Ferritin and reticulocytes were higher in females while haemoglobin did not differ by gender.

Various blood parameters were associated with RET-He. Positive correlations were found between RET-He and parameters reflecting IDA, such as haemoglobin, MCV, MCH, MCHC and reticulocytes. These findings align with a previous study,[14] showing that RET-He changes are consistent with erythropoiesis-related parameters. However, no correlation was found between RET-He and ferritin. Ferritin levels showed a U-shaped curve with age, peaking at 2–6 months and gradually increasing from 6 to 48 months. Relationships between RET-He and iron metabolism biomarkers are complex and vary with age, biomarker type and ID severity. Age-specific analysis revealed no correlation between RET-He and ferritin in 12-month-old infants while a positive relationship was observed in 4-month-old infants, and a consistent negative association with transferrin saturation (TSAT) was found at both ages.[21] Analysis in American children aged 6 months to 18 years revealed that RET-He significantly correlates with ferritin, TSAT and soluble transferrin receptor, making RET-He a potential marker for monitoring erythropoiesis.[25] Another paediatric-population-based study did not demonstrate a linear relationship between RET-He and ferritin, indicating that red cells may reach maximum haemoglobin levels while ferritin continues to rise; RET-He may remain constant until ID is evident.[26] The varying results suggest that different iron metabolism biomarkers could be used to describe different ID stages.

RET-He is an early indicator of ID in children as well as a supporting diagnostic tool in diagnosing IDA without an extra iron metabolism test, especially in inflammation status. RET-He reflects iron availability within a 4-day time frame,[27] especially in predicting the absence of bone marrow iron stores.[28 29] Studies focusing on children have demonstrated the RET-He in identifying ID before the onset of anaemia.[30] Rescreening during the second year of life revealed a ninefold greater risk of developing IDA among children aged 9–12 months with an RET-He<27.5 pg and no initial anaemia.[16] RET-He separated ID cases from the control group with comparatively high sensitivity and demonstrated improved diagnostic performance in IDA group and ID without anaemia.[19] Unlike ferritin increasing in acute phase of diseases,[31] RET-He has a higher specificity and a lower coefficient of variation, it is more appropriate to screen ID and IDA accompanied by inflammatory responses.[32] It has been reported as a sensitive marker of body iron status to conventional tests for the detection of ID in children accompanied with inflammation.[33] Combined with low haemoglobin levels, RET-He is an accurate diagnostic test for ID and IDA in ill infants and children.[34] RET-He is also important in iron treatment monitoring, serving as an early response to iron therapy within days after initiating the treatment.[35] Nevertheless, the use of RET-He in reflecting the effectiveness of iron supplements in IDA children warrants further study.

In our study, the results indicated that the majority of suspected thalassaemia cases had an RET-He value ≤27.8 pg, which is the cut-off determined from the ROC analysis for screening ID. The finding suggests that CHr level could serve as a diagnostic indicator for both thalassaemia and ID. Low MCV and high RCC in

CBC may indicate the presence of a thalassaemia trait, requiring further investigation through a haemoglobin study. RET-He values below the cut-off suggest ID, and measuring ferritin level is advisable for confirmation and further evaluation. Another potential application of RET-He is the differentiation of haematological conditions. Thalassaemia, another microcytic, hypoanaemia, demonstrates a similar change of blood parameters as an ID in CBC.[36] Reduced RET-He can also be observed in haemoglobinopathies like α-thalassaemia and β-thalassaemia, which may not be related to ID. Clinical history and genetic testing are crucial for ruling out haemoglobinopathies. Studies show that patients with β-thalassaemia have lower RET-He levels and a smaller percentage of microcytic reticulocytes compared with those with ID.[28 37] RET-He testing makes it easier to differentiate between the two conditions compared with traditional assessments, allowing for more accurate and timely identification.[28] To distinguish between individuals with thalassaemic traits and early ID, various factors can be considered, such as the micro/hypo ratio, Mentzer index and an algorithm using RET-He, Hb/RET-He ratio and the microratio/hyporatio.[38 39]

RET-He is also advantageous in clinical practice since it is cost-effective and less invasive. Though the conventional iron biomarker-TSAT is also not influenced by inflammation,[33] an extra tube of blood and even a second blood draw are required. The traditional test of ferritin and TSAT parameters costs two times more than the cost of getting CBC but the RET-He can be easily obtained or measured in the same blood tube used in CBC analysis with a specific instrument.[25 40] In our study, RET-He is measured by the Sysmex XN-9100/XN-1500 haematology analyser, which is widely used throughout Hong Kong, thus promoting the feasibility of adopting age-specific reference value of RET-He in initial ID screening in young children.

One notable strength of this study is a large representative sample stratified by sampling from MCHCs and kindergartens across the territory of Hong Kong. Several limitations should be considered. First, this study was cross-sectional, and causality could not be established. Second, this study only included healthy children, so the reference ranges established may not be generalisable to children with diseases that may affect iron status. As we did not measure inflammation biomarkers, participants with inflammatory status cannot be excluded. Third, this study only included children from Hong Kong so the reference ranges established may not be generalisable to other populations with different genetic and environmental factors.

CONCLUSION

This study established reference values for RET-He in healthy children under 5 years old in Hong Kong and explored its relationship with various blood parameters, enhancing our understanding of RET-He in different populations. RET-He can aid in assessing children's iron usage and erythropoiesis, and early identification and treatment of IDA are essential to preventing long-term developmental damage. Longitudinal studies are necessary to determine how RET-He reference levels can predict future IDA in Hong Kong children. More research on RET-He in various populations and its association with iron status is also required. Understanding how RET-He responds to iron treatment in children should be a focus.

Acknowledgements The authors would like to thank the support from the Family Health Service of the Department of Health as well as all participants in this study.

Contributors Concept or design: CCS and PI; Acquisition of data: YTC; Analysis or interpretation of data: KTST; Drafting of the article: HKS, KTST and CC; Critical revision for important intellectual content: KTST, HKS, CCS and PI. PI is the guarantor.

Funding This work is supported by the Hong Kong Health and Medical Research Fund (reference No: Vit D-HKU) and the Collaborative Research Fund, University Grants Committee (Reference No: C7149-20GF).

Competing interests none.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster Research Ethics Committee (UW 13-055, UW 17-491). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iDs
Hung Kwan So http://orcid.org/0000-0002-6188-7587
Patrick Ip http://orcid.org/0000-0002-6797-6898

REFERENCES

1 Gedfie S, Getawa S, Melku M. Prevalence and Associated Factors of Iron Deficiency and Iron Deficiency Anemia Among Under-5 Children: A Systematic Review and Meta-Analysis. *Glob Pediatr Health* 2022;9:2333794X221110860.
2 Safiri S, Kolahi A-A, Noori M, *et al*. Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: results from the Global Burden of Disease Study 2019. *J Hematol Oncol* 2021;14:185.
3 Zhao J, Chen S, Wan R, *et al*. Status of malnutrition and its influencing factors in children under 5 years old in Guangnan District of Yunnan Province in 2009 - 2010. *Wei Sheng Yan Jiu* 2013;42:67–71.
4 Likhar A, Baghel P, Patil M. Early Childhood Development and Social Determinants. *Cureus* 2022;14:e29500.
5 East P, Doom JR, Blanco E, *et al*. Iron deficiency in infancy and neurocognitive and educational outcomes in young adulthood. *Dev Psychol* 2021;57:962–75.
6 Pivina L, Semenova Y, Doşa MD, *et al*. Iron Deficiency, Cognitive Functions, and Neurobehavioral Disorders in Children. *J Mol Neurosci* 2019;68:1–10.
7 McCann S, Perapoch Amadó M, Moore SE. The Role of Iron in Brain Development: A Systematic Review. *Nutrients* 2020;12:2001.
8 Ning S, Zeller MP. Management of iron deficiency. *Hematology Am Soc Hematol Educ Program* 2019;2019:315–22.
9 Hung SC, Tarng DC. Bone marrow iron in CKD: correlation with functional iron deficiency. *Am J Kidney Dis* 2010;55:617–21.

10 Stein J, Bager P, Befrits R, *et al*. Anaemia management in patients with inflammatory bowel disease: routine practice across nine European countries. *Eur J Gastroenterol Hepatol* 2013;25:1456–63.
11 Mast AE, Blinder MA, Lu Q, *et al*. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. *Blood* 2002;99:1489–91.
12 Urrechaga E, Borque L, Escanero JF. Erythrocyte and Reticulocyte Indices on the LH 750 as Potential Markers of Functional Iron Deficiency. *Anemia* 2010;2010:625919.
13 Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol* 2006;1 Suppl 1:S4–8.
14 Urrechaga Igartua E, Hoffmann JJML, Izquierdo-Álvarez S, *et al*. Reticulocyte hemoglobin content (MCHr) in the detection of iron deficiency. *J Trace Elem Med Biol* 2017;43:29–32.
15 Kiudeliene R, Griniūte R, Labanauskas L. Prognostic value of reticulocyte hemoglobin content to diagnose iron deficiency in 6-24-month-old children. *Medicina (Kaunas)* 2008;44:673–7.
16 Ullrich C, Wu A, Armsby C, *et al*. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. *JAMA* 2005;294:924–30.
17 López-Ruzafa E, Vázquez-López MA, Lendinez-Molinos F, *et al*. Reference Values of Reticulocyte Hemoglobin Content and Their Relation With Other Indicators of Iron Status in Healthy Children. *J Pediatr Hematol Oncol* 2016;38:e207–12.
18 Teixeira C, Barbot J, Freitas MI. Reference values for reticulocyte parameters and hypochromic RBC in healthy children. *Int J Lab Hematol* 2015;37:626–30.
19 Poventud-Fuentes I, Chong TH, Dowlin M, *et al*. Reticulocyte hemoglobin equivalent as a marker to assess iron deficiency: A large pediatric tertiary care hospital study. *Int J Lab Hematol* 2024;46:148–55.
20 Moscheo C, Licciardello M, Samperi P, *et al*. New Insights into Iron Deficiency Anemia in Children: A Practical Review. *Metabolites* 2022;12:289.
21 Löfving A, Domellöf M, Hellström-Westas L, *et al*. Reference intervals for reticulocyte hemoglobin content in healthy infants. *Pediatr Res* 2018;84:657–61.
22 Ziegler EE. Consumption of cow's milk as a cause of iron deficiency in infants and toddlers. *Nutr Rev* 2011;69 Suppl 1:S37–42.
23 McMillen SA, Dean R, Dihardja E, *et al*. Benefits and Risks of Early Life Iron Supplementation. *Nutrients* 2022;14:4380.
24 Kuehn D, Roberts SS, Olsen CH, *et al*. Reticulocyte hemoglobin content testing for iron deficiency in healthy toddlers. *Mil Med* 2012;177:91–5.
25 Neef V, Schmitt E, Bader P, *et al*. The Reticulocyte Hemoglobin Equivalent as a Screening Marker for Iron Deficiency and Iron Deficiency Anemia in Children. *J Clin Med* 2021;10:3506.
26 Perchard M, Barbaro P, Rane M, *et al*. Clinical utility of reticulocyte haemoglobin in the assessment of iron deficiency and iron deficiency anaemia in the paediatric population. *J Paediatr Child Health* 2023;59:153–8.
27 Brugnara C, Adamson J, Auerbach M, *et al*. Iron deficiency: what are the future trends in diagnostics and therapeutics? *Clin Chem* 2013;59:740–5.
28 Ogawa C, Tsuchiya K, Maeda K. Reticulocyte hemoglobin content. *Clin Chim Acta* 2020;504:138–45.
29 Mittman N, Sreedhara R, Mushnick R, *et al*. Reticulocyte hemoglobin content predicts functional iron deficiency in hemodialysis patients receiving rHuEPO. *Am J Kidney Dis* 1997;30:912–22.
30 Shaker M, Jenkins P, Ullrich C, *et al*. An economic analysis of anemia prevention during infancy. *J Pediatr* 2009;154:44–9.
31 Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. *Int Immunol* 2017;29:401–9.
32 Kılıç M, Özpınar A, Serteser M, *et al*. The effect of reticulocyte hemoglobin content on the diagnosis of iron deficiency anemia: A meta-analysis study. *J Med Biochem* 2022;41:1–13.
33 Marković M, Majkić-Singh N, Ignjatović S, *et al*. Reticulocyte haemoglobin content vs. soluble transferrin receptor and ferritin index in iron deficiency anaemia accompanied with inflammation. *Int J Lab Hematol* 2007;29:341–6.
34 Swart PDR, Rautenbach K, Raubenheimer JE. Reticulocyte haemoglobin content as a diagnostic tool for iron deficiency and iron-deficiency anaemia in ill infants and children. *S Afr J CH* 2014;8:23.
35 Gelaw Y, Woldu B, Melku M. The Role of Reticulocyte Hemoglobin Content for Diagnosis of Iron Deficiency and Iron Deficiency Anemia, and Monitoring of Iron Therapy: a Literature Review. *Clin Lab* 2019;65.
36 Jameel T, Baig M, Ahmed I, *et al*. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. *Pak J Med Sci* 2017;33:665–9.
37 Velasco-Rodríguez D, Alonso-Domínguez J-M, González-Fernández F-A, *et al*. Reticulocyte parameters of delta beta thalassaemia trait, beta thalassaemia trait and iron deficiency anaemia. *J Clin Pathol* 2016;69:149–54.
38 Mateos ME, De-la-Cruz J, López-Laso E, *et al*. Reticulocyte hemoglobin content for the diagnosis of iron deficiency. *J Pediatr Hematol Oncol* 2008;30:539–42.
39 Stoffman N, Brugnara C, Woods ER. An algorithm using reticulocyte hemoglobin content (CHr) measurement in screening adolescents for iron deficiency. *J Adolesc Health* 2005;36:529.
40 Chadha V, Mudaliar S, Khurana R, *et al*. Burden of Iron Deficiency and Correlation of Reticulocyte Hemoglobin Content (CHr) in Iron Deficiency-a Study from Tertiary Care Center in Developing Country. *Blood* 2022;140:11070–1.