AMJ Australasian Medical Journal [AMJ 2021;14(7):183-189]

Significance of reticulocyte haemoglobin content in diagnosis of iron deficiency anaemia

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RESEARCH

Please cite this paper as: Shah, N. Pathak, J. Shah, P.Signiicance of reticulocyte haemoglobin content in diagnosis of iron deficiency anaemia. AMJ 2021;14(7):183–189. https://doi.org/10.35841/1836-1935.14.7.183-189

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ABSTRACT

Background
Reticulocyte haemoglobin content (CHr) measures the amount of haemoglobin in reticulocytes and hence, offers real-time information on iron supply for erythropoiesis. It is a useful diagnostic test for iron deficiency anaemia.

Aims
Our aim in this study was to evaluate the clinical significance of reticulocyte haemoglobin (RET-He) in IDA while also comparing it with currently available parameters.

Methods
This was an observational cross-sectional study conducted in a tertiary care hospital over ten months and included all patients (N=50) above eighteen years of age freshly diagnosed with anaemia. Following detailed history, general examination and basic haematological investigations, the serum iron profile and reticulocyte haemoglobin of patients were assessed. Correlation analysis and comparison of coefficient of variance were done between different parameters of IDA.

Results
There is a significant positive correlation of RET-He with ferritin in total patients (r=0.2121, p=0.1391) as well as with haemoglobin (r=0.3116, p=0.0267), mean corpuscular volume (r=0.1822, p=0.0267), serum iron (r=0.1519, p=0.2923), and a significant negative correlation with red cell distribution width (r=0.03029, p=0.8346) and total iron binding capacity (r=-0.2722, p=0.0559). Variation of Ret-He in our study is 34 per cent which is lower than that of haemoglobin (44.2 per cent), serum Iron (68 per cent), serum transferrin saturation (66.8 per cent), and serum ferritin (38.2 per cent).

Conclusion
Ret-He is now called as the gold standard for diagnosis of iron deficiency anaemia surpassing previously used parameters like bone marrow iron and serum ferritin.

Key Words
Reticulocyte haemoglobin content, iron deficiency anaemia, diagnosis

What this study adds:

1. What is known about this subject?
Reticulocyte haemoglobin (Ret-He) is a reliable, cheap, and convenient investigation for the diagnosis of iron deficiency anaemia (IDA).

2. What new information is offered in this study?
Reticulocyte haemoglobin (Ret-He) has a sensitivity and specificity comparable to bone marrow iron content and better than serum ferritin and total iron binding capacity (TIBC).

3. What are the implications for research, policy, or practice?

Ret-He can easily be incorporated into routine screening of IDA as it avoids the use of expensive investigations and also, its values change quickly during follow-up after iron supplementation.

Background

Anaemia is a reduction in the total amount of red blood cells (RBCs) or haemoglobin (Hb) in the blood, or a lowered ability of blood to carry oxygen.[1-3] Iron deficiency anaemia (IDA) is the leading cause of anaemia worldwide and especially India along with being one of the commonest nutrient deficiencies.[4] WHO (World Health Organisation) criteria for diagnosing IDA consist of Hb<12gram/decilitre (females), Hb<13gram/decilitre (males), and mean corpuscular volume (MCV) <80 femtolitre (with previously documented normal MCV). Iron profile diagnostic of IDA includes serum iron <7.1microgram/litre, serum ferritin <30 nanogram/litre (within six months of visit), serum transferrin saturation (TSAT) <15 per cent, and total iron binding capacity (TIBC) >13.1micromole/litres.[5]

The routinely used laboratory investigations used to diagnose IDA include haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), peripheral smear, serum ferritin, and serum transferrin saturation (TSAT).[4] The differential diagnosis of microcytic hypochromic RBCs includes IDA, anaemia of systemic diseases, thalassemia minor, and sideroblastic anaemia. These can be differentiated on the basis of their iron profile which includes serum iron, total iron binding capacity (TIBC), and serum ferritin. Serum ferritin is considered a good predictor of iron stores. However, its levels rise in the face of inflammation because it is an acute phase reactant.[6-8] Bone marrow iron as measured by Prussian blue staining of the bone marrow aspirate is the traditional gold standard to diagnose iron deficiency anaemia.[9] However, it is an invasive procedure, has high observer variation, and in clinical practice, it is practically never performed.

Today, automated counters play an integral part in the diagnosis of haematological conditions. Along with routine blood parameters, newer generation counters also provide a new parameter - reticulocyte haemoglobin (RET-He). It is a measure of haemoglobin content of the freshly produced red blood cells and thus offers an estimate about the iron stores in bone marrow required for erythropoiesis. Reticulocyte haemoglobin content (CHr) is a measurement of haemoglobin inside the reticulocyte. It correlates directly with the functional availability of iron in the marrow. Today, it is considered the gold standard for diagnosing iron deficiency. It is more sensitive and accurate than serum ferritin as it is not an acute phase protein.[10]

This study is an effort to evaluate the clinical significance of reticulocyte haemoglobin content in diagnosis of IDA while also comparing this diagnostic parameter with the currently available parameters like RBC indices, serum iron, TIBC, TSAT and serum ferritin.

Method

Settings and subjects: This was an observational cross sectional study conducted in Medicine in-patient department of Baroda Medical College and SSG Hospital, Vadodara over a period of ten months, February, 2018 to November, 2018 and included all patients (N=50) above eighteen years of age of either gender who were freshly diagnosed on admission with microcytic hypochromic or normocytic normochromic anaemia and gave a written informed consent. Haemoglobin levels less than 12gram/decilitre taken as cut-off for anaemia in females and less than 13gram/decilitre for males. Exclusion criteria consisted of patients with anaemia secondary to acute blood loss, macrocytic anaemia, and anaemia with dimorphic picture as diagnosed by peripheral smear at the pathology department of our set-up. Patients on haematinics and pregnant females as elicited by history were also excluded.

Recruitment methods: After obtaining approval from the institutional ethics committee (ECR/85/Inst/GJ/2013/RR-16) and taking a well-informed written consent, all participants were subjected to standardised interview which consisted of demographic data, symptoms of generalised weakness, easy fatigability, dyspnoea on exertion, palpitation, pedal oedema, yellow discolouration and diarrhoea, menstrual and obstetric history for females, past history for major illnesses, and personal history including diet, appetite, sleep, bowel and bladder movements, and addiction. Clinical examination of patients done for findings pertaining to anaemia such as poor nourishment, tachycardia, pallor, koilonychia, etc. Complete blood count including haemoglobin (Hb), total leukocyte count (TLC), platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) was performed by optical measurement method on Horiba haematology analyser (Benaka Healthcare, France). Erythrocyte sedimentation rate (ESR) by Wintrobe method, random blood sugar (RBS) by glucose oxidase-peroxidase method, and bilirubin (total, direct, indirect) by diazotized sulfanilic acid were performed in Robonik Prietest Touch Biochemistry Analyser. Stool examination by light microscope and electrocardiogram(ECG) were also done.

After initial assessment, those patients having low Hb, reduced MCV, reduced MCH, reduced MCHC, and peripheral smear suggesting hypochromic microcytic or normocytic normochromic anaemia on the haemogram were enrolled in the study.

Diagnostic tests: The severity of anaemia was classified according to the WHO criteria which states that mild anaemia is with Hb between 11 to 11.9g/dl in females and 11 to 12.9g/dl in males, moderate with Hb between 8 to 10.9g/dl and severe anaemia with Hb less than 8g/dL.[11] Serum iron profile consisting of serum iron, TIBC, serum ferritin, and TSAT along with absolute reticulocyte count, reticulocyte percentage, and reticulocyte haemoglobin (RET-He) were sent for analysis. These blood parameters were measured in XN – 350 Sysmex, a six-part haematology auto-analyser which works on the principle of flow cytometry with laser side and forward method.

Sample size: Sample size (N=50).

Data collection and analysis: All the data was analysed using appropriate statistical tests. A p value of <0.05 was considered significant assuming normal distribution of dependent variables and randomisation of independent variables. Qualitative data was expressed in percentage and quantitative data was expressed as mean±standard deviation. Correlation analysis was done by using Pearson correlation. Comparison of coefficient of variance was done between different parameters of IDA. Data was entered with the help of Microsoft Word and Excel and analysed by MedCalc Software Version 12.5.0 (Osterd, Belgium).

Results

Majority of our cases belonged to the age group of 18–50 years which comprised of 42 cases (i.e., 84 per cent of all cases). Out of 50 patients, 37 were females (i.e., 74 per cent) and 13 were males (i.e., 26 per cent). Except for the age group of 61–70 years, females were predominant across all age groups. Maximum number of female cases belonged to the age group of 18–30 years with 17 cases (i.e., 34 per cent of all cases) followed by 31–50 years comprising of 15 cases (i.e., 30 per cent of all cases).

(Figure 1) In this study, there were 45 cases (i.e., 90 per cent of all cases) with severe anaemia, 5 cases (i.e., 10 per cent of all cases) with moderate anaemia and no case with mild anaemia. Out of 13 male patients, 2 had moderate anaemia and 11 had severe anaemia while out of 37 female patients, 3 had moderate anaemia and 36 had severe anaemia.

The haemoglobin concentration of patients in this study varied from 1.3 to 9g/dl with a mean value of 4.882±2.155g/dl. MCV and the MCH values were lower than the normal range of 79 to 95fl and 27 to 31pg respectively whereas the RDW values were higher than normal (11.5 to 16 per cent).

The range of serum iron values in our study was 3 to 71mcg/dl with a mean value of 26.404±17.964mcg/dl. The range of TIBC in this study was from 224 to 613mcg/dL with a mean value of 423.462±88.23mcg/dL. The serum ferritin values had a range from 1.66 to 31.77ng/dl with a mean value of 12.5532±8.39ng/dl. The range of transferrin saturation in the current study was from 1.83 to 10.98 per cent with a mean of 7.18±2.746 per cent.

The Reticulocyte haemoglobin content (CHr) in this study had a range from 1.06 to 25.8pg with a mean Ret-He of 13.9192±4.597pg. The range of Absolute reticulocyte count was from 10706 to 108053/microlitre with a mean value of 48205±20573/microlitre. The range of Reticulocyte percent was from 0.53 to 5.17 per cent with a mean value of 1.6174±1.08 per cent. The range of immature reticulocyte fraction was from 11.9 to 49.8 per cent with a mean value of 23.9 ±9.88 per cent.

We have observed significant positive correlation of RET-He with ferritin in total patients (r=0.2121, p=0.1391) as well as with Hb (r=0.3116, p=0.0267), with MCV (r=0.1822, p=0.0267), with serum iron (r=0.1519, p=0.2923) and a significant negative correlation with RDW (r=-0.03029, p=0.8346) and TIBC (r=-0.2722, p=0.0559). Hence with decrease in Hb, MCV, serum iron and increase in RDW and TIBC there will be a decrease in Ret-He value. There is also a significant positive correlation between serum ferritin with Hb (r=0.2215, p=0.1221), with MCV (p=0.4290, r=0.0019) and with serum Iron (p=0.2557, r=0.0730) and significant negative correlation with TIBC (p=-0.1825, r=0.2047). Hence with decrease in Hb, MCV and serum iron and increase in TIBC, there will be a reduction in serum ferritin value.

Mean variation of Ret-He in our study was 34 per cent which is lower than that of haemoglobin (44.2 per cent), serum iron is (68 per cent), TSAT (66.8 per cent), and serum ferritin (38.2 per cent).

Discussion

In the present study, it was found that maximum number of patients belonged to a younger age group however; iron deficiency anaemia is not rare in the older age groups as well. The incidence of iron deficiency was higher in females, irrespective of age. This could be due a high level of

malnutrition, increased loss of menstrual blood in menorrhagia, blood loss during childbirth and depletion of iron stores following pregnancy and lactation. IDA patients had consistently low Hb, MCV, and MCH levels with high RDW values. Hence, peripheral smear in IDA generally shows microcytic hypochromic RBCs with anisopoikilocytosis. Iron profile in patients was suggestive of IDA with low serum iron, low serum ferritin, low TSAT, and high TIBC. We observed a significant positive correlation of RET-He with ferritin in all patients as well as with Hb, MCV, serum iron, and a significant negative correlation with RDW and TIBC. These findings are similar to those with serum ferritin. Hence, Ret-He can be used as a parameter in the diagnosis of iron deficiency anaemia.

The range of serum iron values in our study was 3 to 71mcg/dl with a mean value of 26.404±17.964mcg/dl. This value is higher than values obtained from other studies like Sudhir et al.[4] with a mean serum iron of 19.22±5.59mcg/dl and Ana Beatriz et al.[12] with a mean serum iron of 24.5mcg/dl with a range of 7 to 73mcg/dl. Sudhir et al.[4] had a mean TIBC of 429.32±36.07mcg/dl which is higher than the mean value of TIBC in our study (423.462±88.23mcg/dl). The serum ferritin values had a range from 1.66 to 31.77ng/dl with a mean value of 12.5532±8.39ng/dl which is more than the mean serum ferritin obtained by Sudhir et al.[4] (9.97±4.19ng/dl) and Ana Beatriz et al.[12] (8.2ng/dl with a range of 3.2 to 23.4ng/dl). The range of TSAT in the current study was from 1.83 to 10.98 per cent with a mean of 7.18±2.746 per cent. Sudhir et al.[4] had a mean TSAT of 4.51±1.39 per cent and Ana Beatriz et al.[12] had a mean TSAT of 6.1 per cent with a range of 1.5 to 15.5 per cent. The Reticulocyte Haemoglobin content (CHr) in this study had a range from 1.06 to 25.8pg with a mean Ret-He of 13.9192±4.597pg which is lower than previous studies - Sudhir et al.[4] (Ret- He 19.19±4.02 pg specifically in the iron deficiency anaemia group), Ana Beatriz et al[12] (Ret-He 25.2pg with a range of 16.9 to 32.6pg), and Alan E Mast et al.[13] (Ret-He 30.8±0.9 pg with a range of 28.9 to 32.9pg). In a study by Brugnara et al.[14] with RET-HE cut off level of 27.2pg, iron deficiency could be diagnosed with a sensitivity of 93.3 per cent and specificity of 83.2 per cent. Result in my study regarding correlation between different blood parameters is in consistency with previous studies - Sudhir et al.,[4] Mittman et al.,[15] Toki et al,[16] and C. Brugnara et al[14] all of which concluded that Ret-He as well as serum ferritin have a significant positive correlation with Hb, MCV, serum iron and serum ferritin whereas Ret-He has a significant negative correlation with RDW and TIBC.

However, there are some diagnostic limitations of Ret-He as it depends on mean corpuscular volume (MCV). Ret-He is low in subjects with thalassaemia and haemoglobinopathies without iron deficiency as MCV is low in these conditions. Similarly, it is elevated in iron deficient subjects with confounding megaloblastic anaemia because of high MCV. Therefore, it is important that Ret-He is interpreted in the context of patient's overall erythrocyte physiology including co-existing megaloblastic anaemia, thalassaemia, haemoglobinopathies or blood transfusion. Since majority of patients in our study had severe anaemia, whether our findings can be extrapolated to those with mild to moderate degree of anaemia or with iron deficient states is debatable. This study was done at a tertiary care centre and recruited participants from in-patient department preferably resulting in a limited sample size, thus the nature of the investigation and the results do not imply a general case, and further studies with a larger sample size are needed.

The implications of this study are that Ret-He can easily be incorporated into routine screening of IDA along with complete blood count and hence, replace the traditionally used serum ferritin. Ret-He avoids the use of expensive investigations and also, its values change quickly during follow-up after iron supplementation. Further studies on this topic are required to assess its widespread use in large heterogenous population.

Conclusion

RET-He is an accurate, simple and inexpensive method of diagnosing IDA whereas serum ferritin being an acute phase reactant can be elevated during inflammation. The traditional gold standard - bone marrow iron store has the disadvantage of being invasive and not feasible in all patients. Hence, Ret-He is now called as the gold standard for diagnosis of iron deficiency anaemia surpassing previously used parameters like bone marrow iron and serum ferritin.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

FUNDING

None

ETHICS COMMITTEE APPROVAL

The study was approved by the Institutional Ethics Committee for Human Research (IECHR), Medical College and SSG Hospital, Baroda. EC Reg No: ECR/85/Inst/GJ/2013/RR-16

Table 1: Age and sex distribution in iron deficiency anaemia

Age (in years)	No. of patients	Percentage	Female	Percentage	Male	Percentage
18-30	22	44%	17	34%	5	10%
31-40	8	16%	7	14%	1	2%
41-50	12	24%	8	16%	3	6%
51-60	7	14%	4	8%	3	6%
61-70	1	2%	0	0	1	2%
71-80	1	2%	1	2%	0	0
18-80	50	100%	37	74%	13	26%

Table 2: Haematological parameters of study population

Parameter	Mean Value	SD
Hb	4.882g/dl	2.155g/dl
MCV	56.486fl	8.62fl
MCH	16.008pg	4.741pg
MCHC	26.272g/dl	19.6g/dl
RDW	22.98%	3.98%
PCV	17.62%	6.75%

Table 3: Iron profile of study population

Parameter	Mean	SD
serum iron	26.404mcg/dl	17.964mcg/dL
TIBC	423.462mcg/dl	88.23mcg/dL
serum ferritin	12.5532ng/dL	8.39ng/dL
TSAT	7.18%	2.75%

Table 4: Reticulocyte parameters of study population

Parameter	Mean	SD
Reticulocyte Haemoglobin content	13.9192pg	4.597pg
Absolute reticulocyte count	48205 per microliter	20573.489 per microliter
Reticulocyte percent	1.62%	1.09%
Immature Reticulocyte Fraction	23.90%	9.88%

Table 5: Correlation between different blood parameters

	r (Correlation Coefficient)	P
RET-HE with Hb	0.3116	0.0267
RET-HE with MCV	0.1822	0.2053
RET-HE with RDW	-0.03029	0.8346
RET-HE with serum iron	0.1519	0.2923

RET-HE with TIBC	-0.2722	0.0559
RET-HE with serum ferritin	0.2121	0.1391
serum ferritin with HB	0.2215	0.1221
serum ferritin with MCV	0.429	0.0019
serum ferritin with s. Iron	0.2557	0.073
serum ferritin with TIBC	-0.1825	0.2047

Table 6: Comparison of coefficient of variation of different parameters in iron deficiency anaemia

Indicator	Mean	SD	Coefficient of variance
Hb	4.882g/dl	2.155g/dL	44.20%
serum iron	26.404mcg/dL	17.964mcg/dL	68%
TSAT	12.5532ng/dL	8.39g/dL	66.80%
serum ferritin	7.18%	2.75%	38.20%
Ret-He	13.9192pg	4.597pg	34%

Figure 1: Severity of anaemia in study population

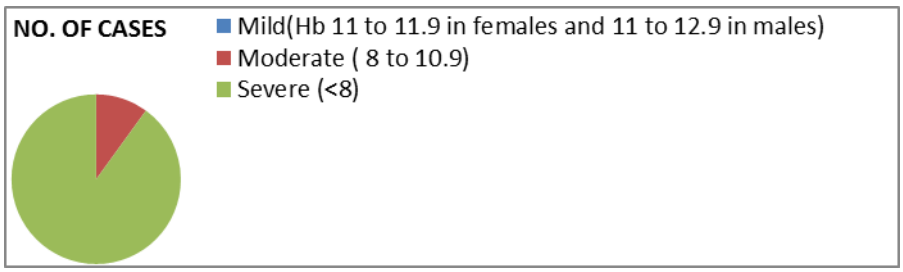

Figure 2: Scatter plot diagram showing the correlation of Ret-He with Serum Ferritin