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# Clinical Significance of Reticulocyte Hemoglobin Content (Ret He) in Diagnosis of Iron Deficiency Anemia

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**KEYWORDS**
Anemia, Erythrocyte indices, Hemoglobins, Iron-Deficiency, Reticulocytes, Reticulocyte hemoglobin content (RET He).

**ABSTRACT:**
Background: Reticulocyte hemoglobin (RET He) measures incorporation of iron into hemoglobin of reticulocytes. This study aims to evaluate the clinical significance of reticulocyte hemoglobin content (RET He) in the diagnosis of iron deficiency anemia (IDA) and to compare it with other conventional iron parameters.
Materials and Methods:
A cross sectional observational study was conducted at Ruby Hall Clinic, Pune. Hemograms of 186 cases were studied on Allinity Hq. RET He was obtained in the same report. Iron studies including serum ferritin, serum iron and total iron binding capacity (TIBC) were done. All hemograms with Microcytic Hypochromic anaemia were included as cases and normal hemograms were taken as controls. Mean ± Standard Deviation and p value of all parameters were calculated. Pearson's correlation coefficient was done. Sensitivity, specificity, PPV and NPV of RET HE with cut off 27 and 28 were calculated.
Results: Out of 186 cases,93 Samples were from healthy controls and 93 were IDA with male: female ratio 0.63. RET He was 23.32±2.49 pg in patients with IDA and 31.87±2.29 pg in controls. RET He showed positive correlation with hemoglobin, erythrocyte indices, serum iron, and transferrin saturation and negative correlation with total iron-binding capacity. With the RET He cut off <27pg and <28 pg; the sensitivity, specificity, PPV and NPV values were 100%,93.9%,93%,100% and 98.9%, 100%, 100%, 90% respectively.
Conclusion: RET He is a simple parameter which predicts IDA accurately, saves on extra draws, additional tests and costs to the patient, besides being prompt.

## INTRODUCTION

Iron deficiency anaemia (IDA) is the most common form of nutritional anaemia worldwide[1]
Though IDA is a treatable condition, it has significant disease burden. Successful management of IDA requires accurate diagnosis followed by investigation of the underlying cause of iron loss and treatment with iron supplementation[2].Various biochemical parameters are used to diagnose IDA, including ferritin, transferrin saturation (TS), serum iron, and mean corpuscular volume (MCV) [3]. Despite the availability of these parameters, their validity for the diagnosis of IDA is still debatable. Serum ferritin, the specific indicator of iron deficiency, is an acute phase reactant and its level is affected by inflammation. Thus, normal ferritin doesn't exclude accompanying IDA as iron is sequestrated in reticuloendothelial system macrophages. TS fluctuates due to the diurnal variation of serum iron, and serum iron levels decrease with infection, inflammation, and malignancy and increase with liver disease[1].
The reticulocyte haemoglobin equivalent (RET He) is a red cell parameter available on the Allinity Hq and other

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haematology analysers and provides a measure of the bioavailability of iron during erythropoiesis[3,4]The RET He is calculated from the reticulocyte haemoglobin concentration and the mean cell volume of the reticulocytes on the Allinity haematology analyser. If iron stores are low, newly formed reticulocytes will have a low haemoglobin content as iron is required for haemoglobin synthesis. The advantage of this parameter is that the reticulocytes have a shorter lifespan (1 - 2 days) than mature red cells and thus provide an early indication of iron deficiency[4]. The complete blood count (CBC) is the most frequently ordered of all laboratory tests and so expansion of the clinical utility of RET He results produced as part of the CBC could be beneficial for patient diagnosis and management[3]**.** This therefore represents an attractive alternative for the diagnosis of iron deficiency in the hospitalised and outdoor patients[5]. We selected the RET He because it is a more stable assay than the others; if testing is delayed the water content of reticulocytes may change, affecting the reticulocyte volume and haemoglobin concentration, but not the haemoglobin content[3].

The study aimed to evaluate clinical significance of reticulocyte haemoglobin content ( RET He) in the diagnosis of Iron deficiency anaemia ( IDA) from a CBC report and to compare it with other conventional iron parameters. RET He cut off was set to diagnose IDA.

**OBJECTIVES:**

- To evaluate clinical significance of reticulocyte haemoglobin content ( MCHr) in the diagnosis of Iron deficiency anaemia (IDA) & iron deficiency (ID) from a CBC report.
- To reduce the cost to the patient by predicting Iron Deficiency & Iron deficiency Anaemia based on MCHr in a CBC report.

**MATERIALS AND METHODS**

A cross sectional observational study was conducted at the Laboratory, Ruby Hall Clinic, Pune over a period of 6 months (during January 2019 to June 2019) to determine the accuracy of RET He in diagnosing IDA. Total 186 cases were studied after obtaining the ethical committee approval. The informed consent was also taken. Sample Size was determined by using standard deviation from previously published study.[5] Considering the expected margin of error d= 0.3 and standard deviation of RET He as 2 pg among iron defecient cases,the calculated sample size was 170.

**1. Study site:**

Laboratory of Ruby Hall Clinic, Pune.

2.**Study population**:

All cases, fulfilling the inclusion and exclusion criteria and admitted or followed up in Ruby Hall Clinic.

**2. Duration of study**: January 2019 to June 2019

**Inclusion criteria**: All hemograms with Microcytic Hypochromic anaemia.

- MCV< 80 fl
- MCH< 27 pg

Iron deficiency anaemia- Serum ferritin < 10 ng/L
Serum Iron- <30 µg/dL
TIBC- > 400 µg/dL

**Exclusion criteria**: Normocytic Normochromic anaemia, Macrocytic anaemia, any hemoglobinopathies, chronic renal failure patients and post transfusion CBCs

**3.Sample size**: 186

**Methods of data collection:**

The blood samples were collected using EDTA vacutainer and analysed with an automated hematology analyser, Allinity Hq. Hemoglobin,red cell indices and RET Hewere recorded. RET He value was determined on Allinity Hq from measurement of light scatter at different angles after isovolumetric sphering of fluorescent dye stained reticulocytes. From the amount of light scattered at the two different angles, the hemoglobin concentration and cellular volume of individual reticulocytes are independently determined.

IRON and TIBC were obtained from “Vitrios”, fully automated biochemistry analyser. (by Pyridyl azo dye method and on “Cobas” by Ferrozine method without deproteinization method.)

Ferritin was obtained by Enhanced Chemiluminescence Immunoassay (ECI) method on Vitrios and by Electrochemiluminescence assay (ECLIA) method on Cobas.

All hemograms with Microcytic Hypochromic anemia. (MCV< 80 fl, MCH< 27 pg), Serum ferritin < 10 ng/L, Serum Iron- <30 µg/dL and TIBC- > 400 µg/dL were included in the study as cases. The cases with normal hemograms were considered as controls. Exclusion criteria were Normocytic Normochromic anaemia, Macrocytic anaemia, any hemoglobinopathies, chronic renal failure patients and post transfusion CBCs

**Statistical analysis and methods:**

Data were collected in a predesigned proforma and later tabulated in a Microsoft excel sheet.

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SPSS software version 20, IBM Corporation was used for data analysis. Results on categorical data were shown as n (% of cases) and the data on continuous measurements were presented as Mean ± Standard Deviation. Karl Pearson's correlation coefficient was used to check correlation between RET He in iron deficient patients and other variables like MCV, MCH ,serum iron etc. Chi square test for qualitative data and independent sample 't' test for quantitative data was applied .P value < 0.05 was considered as statistically significant.

The following formulas were used to calculate the sensitivity, specificity, positive predictive value, negative predictive value.

**Sensitivity** = [True Positive/ (True Positive + False Negative)] × 100

**Specificity =** [True Negative/ (True Negative + False Positive)] × 100

**Positive Predictive Value(PPV)** = [True positive/ (True Positive + False Positive) × 100

**Negative Predictive Value(NPV)** = [True Negative/ (True Negative + False Negative) × 100

**RESULTS:**

A total of 186 cases were included in the study in which, there were 93 cases having microcytic hypochromic anaemia suspected to have iron deficiency anaemia on the basis of CBC report and confirmed with iron profile. The rest 93 cases with normal hemogram and iron profile were taken as control.

There were 89 (47.8%) males and 97 (52.2%) females. IDA was seen in 36 males and 57 females. There was a slight predominance of females with a mean (range) age of 52 years (4 - 89 years). The average age was 52.64 ± 16.25, maximum 91 (48.9 %) patients were in age group 41-60 years and minimum 5(2.7 %) patients were in age group 1-20 years.

The comparison of RET He, erythrocyte indices and iron indices of cases and control was shown in Table I . There was significant difference (p value < 0.05) in all calculated indices between tests of Normal and IDA patients.

The results for the RET He from 186 samples were tested with a mean of 27.5pg and this value we set as a cut off value for present study shown in figure 2 B.

Out of the total 186 cases, 93 were diagnosed as iron deficiency anaemia with RET He cut off 27.5 which is prospectively compared with iron parameters. (mean of 31.5 & 23.3 of normal subjects and IDA patients respectively).

We evaluated the correlation between RET-He, red cell indices and iron parameters, which reflect the hemoglobin content in reticulocyte. There was positive correlation of RET-He with MCV, IRON, FERRITIN, MCHC and negative corelation with TIBC shown in Figure.1

Sensitivity, specificity, positive (PPV) & negative predictive values of RET He for determining IDA were calculated using two cut off values of <27 pg and <28 pg. These findings are summarised in Table II.

**DISCUSSION:**

Microcytic hypochromic anaemia is a frequently encountered problem in daily medical practice. Various biochemical parameters are being used for the diagnosis of IDA. However, there might be some difficulties in the assessment of these conventional parameters. For example, ferritin behaves as an acute phase reactant, which limits its diagnostic accuracy greatly. The serum ferritin level is frequently increased independently of iron status by factors such as acute or chronic inflammation, infection, malignancy, liver disease, and alcohol use. Serum iron levels also decrease with infection, inflammation, malignancy and increase with liver disease. TS is a calculated parameter, and therefore it reflects confounding effects on individual components. Measures of mature erythrocyte indices obtained on automated hematology analysers are not sensitive indicators of early iron deficient erythropoiesis because of the slow turnover of erythrocytes (120 days) and broad interindividual variability[4].

The introduction of automated flow cytometric methods has led to the ability to perform accurate quantitative and qualitative analyses of reticulocytes. Reticulocytes are the first red blood cells to enter the circulation, where they rapidly lose their mitochondria and ribosomes, becoming mature red cells over a 24-hour period. Several indices of reticulocyte size and composition can be measured with the new technology such as the mean cell volume (MCVr), mean cell hemoglobin concentration (MCHCr) and the reticulocyte hemoglobin content (RET He ).

IDA has a very strong socio-economic impact and, diagnosis is usually based on expensive tests. The gold standard for the assessment of body iron is a bone marrow biopsy but it is an invasive procedure rarely used in the diagnosis of ID related disorders[4]. Hence there is a need for rapid and low-cost screening tests allowing the correct diagnosis and the most appropriate management.

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The clinical usefulness of measuring hemoglobin content in reticulocytes has been demonstrated in the identification of iron deficiency and IDA in adults and children, with the advantage of a non-invasive method of measuring current iron stores in bone marrow[6].

The present study showed slightly more prevalence of IDA in females with the mean age of 52 years which was comparable with the study done by Jie Cai et al[7].

The iron deficient group had significantly lower Hb, MCV and MCH values (P<0.001for all) compared with the healthy group. Interestingly, no significant differences could be found in plasma ferritin (P=.97). A marked difference was also noted in the values of RET He, which was significantly decreased in the iron deficient group(P<0.001).This was comparable with the study by Jie Cai et al[7].

Scatterplots showed good correlation between RET He and MCV, MCHC, IRON &FERRITN.

Out of the total 186 cases, 87 were diagnosed as iron deficiency anaemia and 06 cases were false positive which had anaemia due to other causes with RET He cut off 27 which is prospectively compared with iron parameters. With the RET He cut off 27.5pg the sensitivity, specificity, PPV and NPV values are 100%. In order to establish unbiased results, we have taken two different cut off values. Results indicated that a RET He cut off of <27pg was able to identify ID patient groups and best supported the use of the RET He as a screening test for iron deficiency[4] which was similar to an earlier study by Brugnara et al[8]using similar methodologies, demonstrated that RET He was superior to ferritin, transferrin saturation, and serum iron in detecting ID with a sensitivity of 100%, a specificity of 93.9%,PPV of 93% and NPV of 100%.

With the RET He cut off <28pg the sensitivity, specificity, PPV and NPV values are 98.9%,100%,100% and 90% respectively.

Results showed that a cut off of <27pg had better advantage for the detection of IDA over cut off of <28 pg. Charlotte Poffenroth et al[3]study also revealed similar findings**.**

**Limitation:** The RET He is measured by few haematology analysers, hence the availability this parameter is laboratory specific.

The future acceptance of the value of the RET He by clinical staff may be hampered by a lack of awareness of its potential for patient diagnosis and treatment. The education of clinical staff could start by reporting the parameter in anaemic patients with results below the RET He cut off, triggering a comment about additional laboratory testing to rule out possible ID

**CONCLUSION:**

Reticulocyte hemoglobin content (RET He) is an extremely valuable recent addition to an expanding list of biomarkers that can be used to differentiate iron deficiency from other causes of anaemia. The study showed that the RET He with a cut off of <27 pg was highly sensitive.

RET He is a helpful parameter which reduces extra draws, turnaround time and cost for the patient.

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**CONFLICT OF INTEREST STATEMENT:**

The authors of this paper have no conflicts of interest, including specific financial interests, relationships and /or affiliations relevant to the subject matter or materials.

**REFERENCES:**

1. Karagülle M, Gündüz E, SahinMutlu F, Olga Akay M. Clinical significance of reticulocyte hemoglobin content in the diagnosis of iron deficiency anemia. Turk J Haematol. 2013;30:153–156.
2. Schapkaitz E, Buldeo S, Mahlangu JN. A prospective evaluation of the reticulocyte haemoglobin content (CHR) at the Charlotte Maxeke Johannesburg Academic Hospital. Medical Technology SA. 2016 Dec; 30:15-8.
3. Poffenroth C, Mabbett C, Kendrick C. The reticulocyte haemoglobin equivalent (RET_He) and laboratory screening for iron deficiency. N Z J Med Lab Sci. 2017 Nov; 71:120-123.
4. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content.Am J Hematol.2008 Apr ;83 :307-10.
5. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte haemoglobin content in the evaluation of iron status of hemodialysis p atients.Kidney Int. 1997 Jul;52:217-22.
6. Jarc E, Zupan IP, Ponikvar JB, Snoj N, Podgornik H. Comparison of erythrocyte and reticulocyte indices for the diagnosis of iron defciency. ZdravVestn. 2017 Jan- Feb;86:19-27.

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7. Cai J, Wu M, Ren J, Du Y, Long Z, Li G, et al. Evaluation of the efficiency of the reticulocyte hemoglobin content on diagnosis for iron deficiency anemia in Chinese adults. Nutrients. 2017 May 2;9.pii:E450.
8. Brugnara C. Use of reticulocyte cellular indices in the diagnosis and treatment of hematological disorders. International Journal of Clinical and Laboratory Research. 1998 Mar 1;28(1):1-1.
9. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. Jama. 1999 Jun 16;281(23):2225-30.
10. Koepke JF, Koepke JA. Reticulocytes. Clinical & Laboratory Haematology. 1986 Sep;8(3):169-79.
11. Means RT, Glader B. Wintrobe's Clinical Hematology. Wintrobe's Clinical Hematology. 2009:779-809

**Tables and figure**

**Table-I: Comparison of hematologic and biochemical variables between Normal and IDA**

| **Tests** | **Mean±SD** | | **P Value** |
|---|---|---|---|
| | Normal | IDA | |
| **RET He (**pg) | 31.87 ± 2.29 | 23.32 ± 2.49 | 0.0001 |
| **IRON (**ug/dl) | 120.22 ± 109.12 | 23.02 ± 6.61 | 0.0001 |
| **Ferritin (ng**/dl) | 55.62 ± 41.79 | 7.10 ± 2.18 | 0.0001 |
| **MCV (**fl) | 91.57 ± 8.41 | 69.86 ± 10.56 | 0.0001 |
| **MCHC (**g/dl) | 32.10 ± 1.80 | 31.47 ± 1.70 | 0.011 |
| **TIBC** (ug/dl) | 341.56 ± 317.03 | 470.37 ± 36.47 | 0.0001 |
| **MCH (**pg) | 29.65 ± 2.72 | 22.28 ± 2.95 | 0.0001 |
| **Hb (**gm/dl) | 12.18 ± 2.60 | 8.67 ± 2.36 | 0.0001 |
| % **Transferrin Saturation (**ug/dl) | 37.95 ± 27.01 | 4.89 ± 1.49 | 0.0001 |

**Table II: Summary of sensitivity, specificity, positive (PPV) & negative predictive values (NPV) for two cut-off values.**

| **RET He** | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
|---|---|---|---|---|
| < 27 pg | 100 % | 93.9 % | 93 % | 100% |
| < 28 pg | 98.9 % | 100% | 100 % | 90% |

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**Figure-1. Scatterplot of RET He (MCHr) vs IRON, FERRITIN, MCV, MCHC showing positive co relation.**

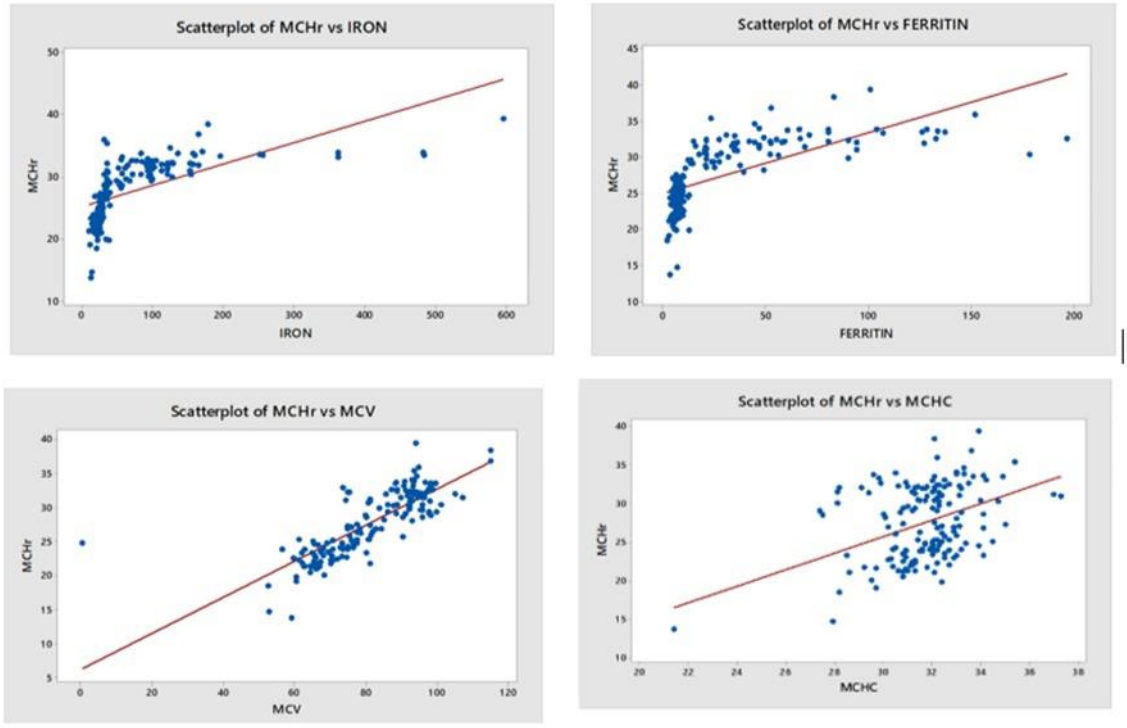


**Figure-2. A. Scatterplot of RET He (MCHr) vs TIBC showing negative corelation. B. A histogram Plot of RET-He with normal distribution curve.**

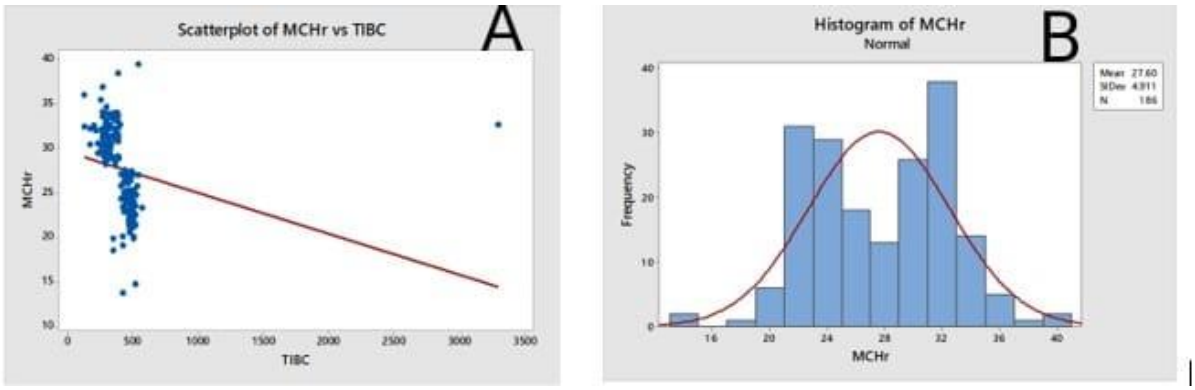


Table-I: Comparison of hematologic and biochemical variables between Normal and IDA
Table II: Summary of sensitivity, specificity, positive (PPV) & negative predictive values (NPV) for two cut-off values.
Figure-1. Scatterplot of RET He (MCHr) vs IRON, FERRITIN, MCV, MCHC showing positive co relation.
Figure-2. A. Scatterplot of RET He (MCHr) vs TIBC showing negative corelation.
B. A histogram Plot of RET-He with normal distribution curve.