

## Reticulocyte Hemoglobin Content (CHr): A Reliable indicator in Iron Deficiency Anemia

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### Abstract

**Introduction:** To estimate a cut off for Reticulocyte Hemoglobin Content (CHr) and compare it with Serum Ferritin for diagnosis of Iron Deficiency Anemia (IDA). **Material & Methods:** A total of 188 samples were included in the analytical study out of which 126 were categorized as study samples and 62 as control. CBC and Reticulocyte analysis were carried out and the parameters were compared. **Results:** CHr has a better sensitivity and specificity compared to Serum Ferritin. Low CHr is a marker for early diagnosis of iron deficiency whereas a raised CHr is an early sign of response to iron therapy. A CHr cut-off of 28.4 pg was established which is comparable with that of other studies. **Conclusion:** The study demonstrates that CHr is a useful parameter that can be confidently used in the diagnosis of IDA.

**Keywords:** Reticulocyte Hemoglobin Content (CHr), Iron Deficiency Anemia (IDA), ROC curve

### Introduction

Anemia is a serious global public health problem, iron deficiency being the most common type of anemia resulting due to nutritional deficiency.<sup>1</sup> Without enough iron, the hemoglobin in the red blood cells is not produced in sufficient amounts which help in carrying oxygen. The signs and symptoms of iron deficiency anemia include: extreme fatigue, weakness, pale skin, chest pain, faster heartbeat or shortness of breath, dizziness, headache, cold hands and feet, inflammation or soreness of tongue, brittle nails and poor appetite.<sup>1</sup> Numerous peripheral blood tests are performed to diagnose iron deficiency, including Serum Iron, Serum Ferritin, Transferrin Saturation and Mean Corpuscular Volume (MCV). Serum Ferritin, the most specific indicator of iron deficiency, is also an acute phase reactant and hence, its level is affected by inflammation.<sup>2</sup>

Reticulocytes are newly produced, relatively immature red blood cells (RBCs) which circulate for 1-2 days in blood before becoming mature reticulocytes. Reticulocyte Hemoglobin Content (CHr) reflects the amount of iron available for hemoglobin production in the bone marrow.<sup>2</sup> Hence, CHr is an indicator which can contribute in prompt diagnosis of IDA and is more reflective of real time Hb synthesis as proven in multiple studies.

### Aims and objectives

1. To estimate a cut off for Reticulocyte Hemoglobin Content (CHr) for diagnosis of Iron Deficiency Anemia (IDA).
2. To deduce the clinical significance of CHr in diagnosis of IDA.
3. To compare CHr with Serum Ferritin.

### Material and Methods

It is an analytical study carried out for duration of 4 months from September 2021 to December 2021 consisting of 188 samples. The samples were categorized into study group (IDA suspected individuals-126 samples) and control group (healthy individuals-62 samples). Table 1 shows the inclusion criteria for the cases and control.

**Table 1: Inclusion criteria**

Variables	Population under study	Control population
Hemoglobin (Hb)	<11 gm/dL	>11 gm/dL
Hematocrit (Hct)	<36%	>36%
Mean Corpuscular Volume (MCV)	<80 fL	>80 fL
Mean Corpuscular Hemoglobin (MCH)	<27 pg	>27 pg
Mean Corpuscular Hemoglobin Concentration (MCHC)	<31.5 gm/dL	>31.5 gm/dL
Red Cell Distribution Width (RDW)	>15	<15
Peripheral smear	Presence of Anisopoikilocytosis	Absence of Anisopoikilocytosis

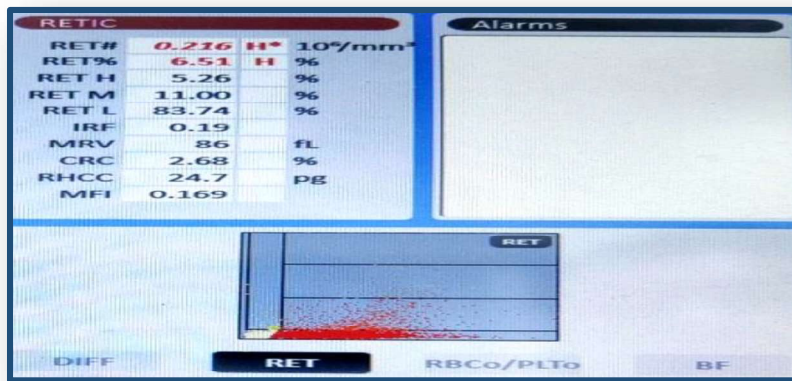
Peripheral blood samples were obtained in EDTA vacuettes for which Complete Blood Count (CBC) and Reticulocyte analysis were carried out using HORIBA Yumizen H2500. It is based on the technique of Fluorescence detection using a RNA-specific fluorochrome (Thiazole orange dye). Leishman stained peripheral smears were examined.

The following RET parameters were analysed: (1) RET#: Reticulocyte Absolute Number (2) RET%: Reticulocyte Percentage (%) (3) RETH%, RETM%, RETL% (4) IRF: Immature Reticulocyte Fraction (5) RHCC: Reticulocyte Hemoglobin Cellular Content (pg) (6) MRV: Mean Reticulocyte Volume (fL) and (7) CRC: Corrected Reticulocyte Count (%). Figure 1 shows the reticulocyte parameters as displayed during the analysis.

**Results**

Out of 188 total samples, 126 were of the study population (IDA suspected) and 62 were of the control population. The mean age of the study population was 42.1 years with age ranging from 17 to 78 years. The mean age of the control population was 35.5 years, with age ranging from 15 to 68 years. Table 2 shows the sex-wise distribution and M:F ratio of the study and control groups.

**Figure 1: Reticulocyte Analysis**



**Table 2: Sex-wise distribution**

	Male	Female	M:F Ratio
Study group	59	67	0.8:1
Control group	34	28	1.2:1
Total	93	95	0.9:1

Table 3 shows the comparison of Hematological and Biochemical Parameters in the study and control populations.

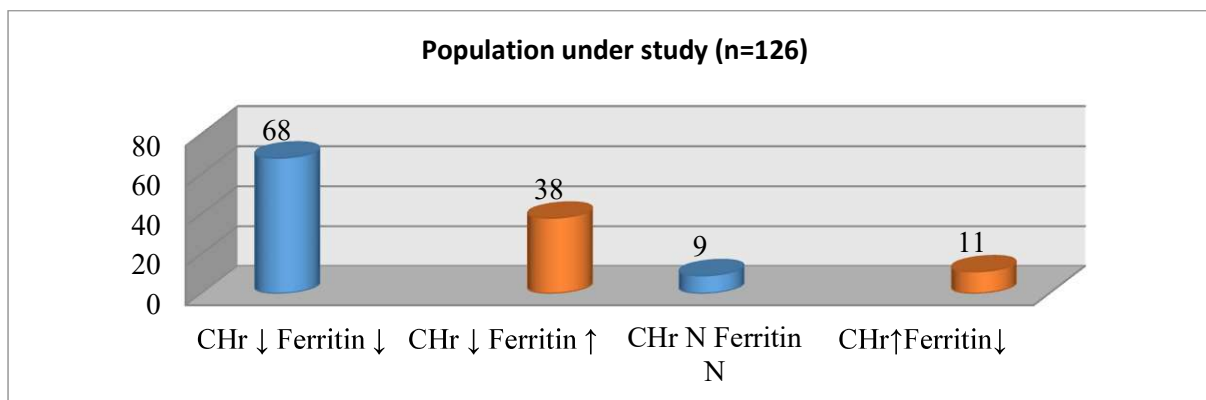
**Table 3: Comparison of Hematological and Biochemical Parameters**

Parameters	Study Population (IDA)		Control Population	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Hemoglobin (gm/dL)	3.9-10.7	7.8 $\pm$ 1.9	11.2-14.8	12.7 $\pm$ 1.4
RBC ( $10^6$ /cumm)	0.9-3.9	2.1 $\pm$ 0.6	3.8-5.5	4.3 $\pm$ 0.4
MCV (fL)	46.7-79.2	72.5 $\pm$ 8.1	81-104	91.1 $\pm$ 7.3
MCH (pg)	12.9-27	22.3 $\pm$ 3.6	27.3-37.8	30.7 $\pm$ 2.9
MCHC (gm/dL)	25.9-31.5	28.7 $\pm$ 1.4	29.8-34.6	32.4 $\pm$ 1.4
Hematocrit (%)	9.9-35.5	25.5 $\pm$ 5.7	36.4-46.9	40.1 $\pm$ 2.9
RDW	15.3-36.8	22.1 $\pm$ 5.6	11.4-15	13.5 $\pm$ 1.4
CHr (pg)	14.8-33	25.6 $\pm$ 3.2	29.5-43	33.2 $\pm$ 3.2
Serum Iron ( $\mu$ g/dL)	0.20-76	30.2 $\pm$ 12.8	55-248	72.5 $\pm$ 30.9
Serum Ferritin (ng/ml)	1.3-705	119.7 $\pm$ 98.7	15.8-124.6	78.8 $\pm$ 32.7

**Table 4: Changes in CHr and Serum Ferritin in Study Population**

Parameter	Reduced	Normal	Increased
Serum Ferritin	79	9	38
CHr	106	9	11

**Figure 2: Variation in CHr and Serum Ferritin in Study Population**



Out of 126 patients under study group, 68 patients who had CHr ↓ and Serum Ferritin ↓ were due to iron deficiency anemia. 38 patients having CHr ↓ but Serum Ferritin ↑ were having any underlying inflammation or infection. 11 patients were on hematinics and had CHr ↑ but still the Serum Ferritin was ↓. Remaining 9 patients had both CHr and Serum Ferritin under normal range.

A Receiver Operating Characteristic (ROC) curve is a graphical plot that shows the trade-off between the sensitivity and specificity. Classifiers that give curves to the top-left corner indicate a better performance. The closer the curve comes to the 5-degree diagonal of the ROC space, the less accurate the test. The Area Under Curve (AUC) is an effective and combined measure of sensitivity and specificity that describes the inherent validity of diagnostic tests.

Figure 3: ROC curve for CHr

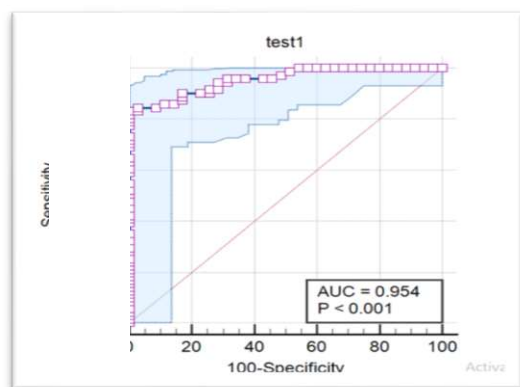


Figure 4: ROC curve for Serum Ferritin

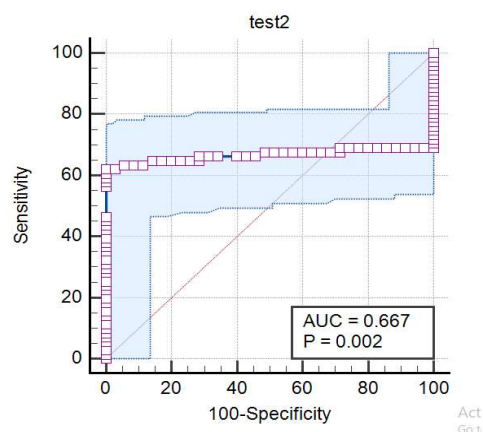


Table 5: ROC Curve Analysis

Indicators	CHr	S. Ferritin
Sensitivity (%)	83.1	61.9
Specificity (%)	100	100
Positive Predictive Value	100	100
Negative Predictive Value	74.5	56.5
Cut-off	≤28.4 pg	<11.9 ng/ml
ROC area	0.954	0.667

### Discussion

Various biochemical parameters are used for the diagnosis of IDA. However, some difficulties arise in the assessment of these conventional parameters. For example, ferritin is an acute phase reactant which limits its diagnostic accuracy greatly. The levels of Serum Ferritin are increased independently of iron status by factors such as acute/chronic inflammation, infection, malignancy, liver disease and alcohol use. Serum Iron levels also decrease with infection, inflammation and malignancy whereas they increase with liver disease. Transferrin saturation is a calculated parameter and hence reflects confounding effects on individual components. Thus, the utility of conventional iron indices in clinical practice at times lead to erroneous results due to biological variability such as diurnal variation, fluctuation with dietary intake, and as acute phase reactants, alteration in inflammatory states.<sup>3</sup>

Hemoglobin concentration assessed using mature erythrocytes do not accurately reflect the real-time iron status due to their long life span. Measurement of CHr provides an indirect measure of the functional iron available for new RBC production over the previous 3-4 days.<sup>4</sup> RET-He values ranging from 28 to 36 pg are generally considered normal.

Based on the maturation stage defined by the RNA content of the reticulocytes, they are separated into three categories according to the degree of fluorescence i.e. Low Fluorescence Reticulocytes (LFR), Middle Fluorescence Reticulocytes (MFR) and High Fluorescence Reticulocytes (HFR). The sum of MFR and HFR is called IRF (Immature Reticulocyte Fraction), is an early and sensitive index for erythropoiesis and therefore has been applied to predict early response to hematinics.<sup>5</sup>

There is an increase in hepcidin production in the patients with inflammatory disorders, systemic infections and malignancies. Iron is trapped in reticuloendothelial system (RES) and also poorly absorbed from GI tract leading to functional iron deficiency and in turn resulting in anaemia of systemic disease (ASD). The peripheral smear in ASD sometimes show a microcytic hypochromic anaemia picture and mimics IDA. Also, in the presence of ASD, it is difficult to diagnose underlying IDA. Such patients have high or normal S. Ferritin despite iron deficiency. In this situation, CHr differentiates ID from ASD. This discriminatory power of CHr, both with respect to sensitivity and specificity, is better than MCV and Serum Ferritin.<sup>5</sup> The serum ferritin concentration values range between 15 and 300 ng/ml in men, whereas it is between 15 and 200 ng/ml in women.<sup>6</sup>

While treating anaemia, physicians remain curious to know the response to treatment at the earliest. It helps to confirm that the treatment is on correct line, and also avoids hazards of over treatment. It takes weeks to observe a significant response to hematinics (oral or I.V, Iron, vitamin B12 or folic acid) by looking at hemoglobin (Hb), Packed cell volume (PCV) or Hematocrit and RBC indices. The main reason for late response is the long lifespan of mature RBC. Hence the need for a more sensitive and reliable marker for assessing the response at an early stage arises. Over the last few years, there have been ample of studies suggesting that reticulocyte, the newly produced erythrocyte may be the solution.<sup>5</sup>

Reticulocyte Hemoglobin Content (CHr) is a parameter that estimates hemoglobinization of most recently produced red cells. Low CHr is a marker for early diagnosis of iron deficiency whereas a raised CHr is an early sign of response to iron therapy. Thus, CHr has been used as early response to treatment following Intravenous (IV) iron therapy in diverse patient populations, including pediatrics, geriatrics, pregnancy & chronic kidney disease (CKD).<sup>5</sup>

In the present study, the ROC curve analysis shows that the Area Under Curve (AUC) for CHr (0.954) is more than that of the Serum Ferritin (0.667). The graph plotted for CHr is more towards the upper left corner than that of S. Ferritin. Hence, could be indicative of a better diagnostic accuracy of CHr in comparison to S. Ferritin.

CHr has a high sensitivity and specificity in the diagnosis of IDA and its comprehensive diagnostic efficacy can aid in early evaluation of IDA patients. Unlike biochemical studies, CHr does not require extra tubes of blood to be drawn; CHr is reported as a part of the reticulocyte count by the hematology analyzer used in this study and is provided without any additional cost.

**Table 6: Comparison of present study with study by Jei Cai et al**

Parameters	Present study	Study by Jie Cai, Meng Wu et al <sup>4</sup>
M:F	0.8:1	0.4:1
Mean Age (years)	42.1	45
Mean CHr (pg)	25.6 ± 3.2	23.3 ± 4
Cut-off for CHr (pg)	≤28.4	27.2

### Conclusion

Reticulocyte hemoglobin content (CHr) 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. A screening approach for iron deficiency on reticulocyte analysis is appealing for its consistency in various biological states, direct real-time assessment of iron metabolism, and ease of collection.

### References

1. World Health Organization (WHO) - Anaemia. [https://www.who.int/health-topics/anaemia#tab=tab\\_1](https://www.who.int/health-topics/anaemia#tab=tab_1)
2. 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 Jun;30(2):153-6. doi: 10.4274/Tjh.2012.0107. Epub 2013 Jun 5. PMID: 24385778; PMCID: PMC3878462.
3. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. *Kidney Int.* 1997 Jul;52(1):217-22. doi: 10.1038/ki.1997.323. PMID: 9211366.
4. Cai J, Wu M, Ren J, Du Y, Long Z, Li G, Han B, Yang L. Evaluation of the Efficiency of the Reticulocyte Hemoglobin Content on Diagnosis for Iron Deficiency Anemia in Chinese Adults. *Nutrients.* 2017 May 2;9(5):450. doi: 10.3390/nu9050450. PMID: 28468320; PMCID: PMC5452180.
5. Agarwal MB, Pai S. Reticulocyte Hemoglobin Content (CHr): The Gold Standard for Diagnosing Iron Deficiency. *J Assoc Physicians India.* 2017 Oct;65(12):11-12. PMID: 31556265.
6. Imelda Bates. Chapter 2: Reference Ranges and Normal Values; Normal Reference Values ,Dacie and Lewis Practical Hematology, Twelfth Edition by Elsevier, 2017, Page 8-17, Table 2-1

## Human Papiloma Virus Vaccination: Knowledge, Attitude and Practice Among Medical Students at Tertiary Care Hospital

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### Abstract

**Background:** Cervical cancer is the leading cause of death in India. HPV virus is a major infectious etiological factor of cervical cancer. It has been proven that HPV vaccine, if taken before initiation of sexual life, is highly effective in HPV and cervical cancer prevention. Despite its availability, deaths due to cervical cancer has increased rapidly. Healthcare workers, especially the young medical students can play a significant role in recommendation of HPV vaccination among people. So, this study was conducted to study the knowledge, attitude and practices of HPV infection and vaccination among medical students. **Methodology:** A cross-sectional observational study was conducted in medical students of Narendra Modi Medical College. Three hundred medical students were requested to fill a questionnaire which covered demographic details, knowledge, attitude and practice towards HPV vaccination for cervical cancer prevention. **Results:** 82.66% of students were aware about the HPV infection out of which 71% of the participants knew that cervical cancer can be prevented by HPV vaccine, 66.66% of students aware of the availability and 35% were well aware about the correct schedule/dosage of vaccination. **Conclusion:** Though the knowledge and attitude about the HPV infection and its vaccination is good among the medical students, but the practice of vaccination is poor.

**Keywords:** HPV – Human Papilloma Virus, Knowledge, Attitude, Practice, HPV vaccine, medical students

### Introduction

Cervical cancer is one of the most common cancers in women worldwide. India accounts for one-fifth of the world burden for cancer. The incidence rate of cervical cancer in Indian women is 26.1-43.8%<sup>1</sup> and accounting for more than 74000 deaths of women per year<sup>2,3</sup>.

Carcinoma cervix is one of the few malignancies where an etiological agent has been identified and is totally preventable through vaccination. Natural infections with HPV induce a weak immune response which may not be protective whereas vaccination results in a strong immune response. Though more than 140 types of HPV have been identified only about 40 types are sexually transmitted. Out Of these, two high risk HPV types 16 and HPV 18 are responsible for more than 80% of cervical cancer