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TO STUDY CLINICAL SIGNIFICANCE OF RETICULOCYTE HAEMOGLOBIN CONTENT IN IRON DEFICIENCY ANEMIA

General Medicine

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ABSTRACT

Background- Anemia is foremost health problem worldwide that need total elimination according to United Nation declaration 1997. Approximately 2 billion population suffer from iron deficiency or anemia globally[1]Worldwide (IDA) is the commonest form of nutritional anemia.[2] Various biochemical markers used for diagnosis of Iron deficiency anemia are MCV, ferritin, transferrin saturation (TS) and serum iron [3]. CHr indicates availability of Iron for haemoglobin production in the bone marrow. Therefore, CHr has been proposed as marker of iron status in body [4] Several studies indicated that CHr measurement useful for IDA[5,6] and reliable marker for monitoring iron therapy's efficacy and iron status in body.[7] that's why we needed this study to know the role of CHr in IDA.
Our objectives were to evaluate the level of reticulocyte haemoglobin content in the diagnosis and treatment of iron deficiency anemia
Materials and Methods – A hospital based prospective study was undertaken at our tertiary care centre in the department of General Medicine, MGMs Medical College & Hospital Aurangabad on admitted patient , of patients with newly diagnosed and untreated Iron Deficiency Anemia (mild-moderate) according to WHO criteria confirmed by iron studies were included.
Results – In the study total 96 patients included amongst them 63.5 % were females and majority patients (35.5 %) were in the age group of 31-40 years. Reticulocyte Haemoglobin (CHr) showed a significant positive correlation with haemoglobin (r=0.782; **p<0.05**), MCV (r=0.871; **p<0.05**), MCHC (r=0.685; **p<0.05**), MCH (r=0.879; **p<0.05**), serum iron (r=0.651; **p<0.05**), and % Saturation (r=0.702; **p<0.05**). CHr showed significant negative correlation with TIBC (r=-0.613; **p<0.05**) and Ferritin (r=-0.604; **p<0.05**).
Conclusion – CHr is a useful parameter that can be confidently used in the diagnosis of Iron deficiency anemia (IDA) and a CHr cut-off value predicts IDA. Ferric carboxymaltose was usually well tolerated and had a good impact in the management of iron-deficiency anemia. After iron replacement therapy CHr values increased correlated with haemoglobin and iron level. We cannot replace iron study but CHr can be used for early diagnosis of Iron deficiency anemia and in management of iron deficiency.

KEYWORDS

Haemoglobin, CHr, iron deficiency anemia

INTRODUCTION-

Anemia is a decrease of total erythrocyte mass and can be caused by a different set of pathophysiological processes. Whatever the etiology is, anemia cause decline in oxygen carrying capacity of blood, often manifesting as a vague complex of symptoms clinically, typically fatigue and dyspnea on exertion.

Anemia is classified in 3 categories according to severity by WHO as mild anemia (Hb 9-10.9gm/dl), moderateanemia (Hb7-9 gm/dl) and severe anemia with Hb<7gm/dl.

Haemoglobin (Hb) plays the role of transporting oxygen to the whole body and is the basis for life support. The lifespan of mature erythrocytes is approximately 120 days, mature erythrocyte Hb cannot sensitively reflect Hb synthesis. The Haemoglobin content of reticulocytes (CHr) can possibly reflect the latest Hb synthesis status. Iron, is a main constituent of Haemoglobin(Hb). An essential element for all cells in the human body almost, with around 70% of iron used by the reticuloendothelial system. CHr is considered useful for measuring acute iron metabolism in the body.

Laboratory markers for diagnosis of Iron Deficiency Anemia(IDA) include Complete blood picture including peripheral smear and iron profile (serum ferritin (SF), serum iron (SI), total iron binding capacity(TIBC) transferrin saturation (TS %)).

Iron deficiency anemia can be diagnosed by low MCV,low serum ferritin level and low serum iron level.As reticulocytes exist in the circulation for only 1–2 days, CHr is an initial marker of functional iron deficiency. Thus, CHr can be beneficial in monitoring the initial state of IDA and response to erythropoietin therapy.[9]

Subsequently classical laboratory biomarkers of Iron deficiency exhibit wide biological variability[10], there is need to assess additional novel and reliable markers. The cogency of a screening or diagnostic tool is associated with the disease prevalence and the characteristics of the study population. Many studies have showed CHr measurement is useful for diagnosis of iron deficiency. And good marker for monitoring iron therapy's effectiveness.

OBJECTIVE-

- To evaluate the level of reticulocyte haemoglobin content in the diagnosis of iron deficiency anemia
- To compare effect of iron therapy on reticulocyte haemoglobin content and other iron parameters in iron deficiency anemia.

MATERIALS AND METHODS –

Study design: A hospital based prospective study

Study Duration: 18 months

Study area: The study was done on admitted patient at our tertiary care centre in the department of General medicine, MGMs Medical College & Hospital Aurangabad, N-6, CIDCO, Aurangabad.

Study population: All adult patients with newly diagnosed and untreated Iron Deficiency Anemia (mild-moderate) according to WHO criteria confirmed by iron studies who fulfilled the inclusion criteria.

Inclusion criteria: All adult patients with newly diagnosed and untreated Iron Deficiency Anemia (mild-moderate) according to WHO criteria confirmed by iron studies routinely done.

Exclusion criteria:

- Diagnosed case of Malignancy
- Any inflammatory Disorder.
- Severe anemia
- Other forms of anemia
- Bleeding tendency

Ethical approval: Approval taken from Ethical Committee of MGM Hospital.

Study procedure:

All patient of mild to moderate iron deficiency anemia included in the study .The sample for peripheral blood smear were obtained on the day of the bone marrow examination and sent for routine analysis at the central pathology laboratories, MGM. Reticulocyte analysis including CHr with complete blood count were measured in whole blood which

is collected in K3EDTA with the use of an ADVIA 120 haematologyanalyzer (Bayer Diagnostics, Tarrytown, NY). Requirement of iron calculated and FCM administerd.

Requirement of iron: Body weight (kg) x 2.3 x (15 – patient's haemoglobin, g/dL) + 500 or 1000 mg (for stores)

Dose of carboxy maltose:

HB(g/dl)	BODY WEIGHT <70 KG	BODY WEIGHT >70 KG
>10	1000mg	1500gm
7-10	1500mg	2000mg

Total iron dosage was administered in single infusion or 1000 mg iron as ferric carboxymaltose

A total cumulative dose 1500mg of iron IV per course is not exceeded. Repeat reticulocyte haemoglobin content, iron parameters, hemogram after 5 days of treatment investigated and result were analysed.

Operational definitions:

1. Anemia- a decrease of total erythrocyte mass and can be caused by a different set of pathophysiological processes. Whatever the etiology is, anemia cause decline in oxygen carrying capacity of blood, often manifesting as a vague complex of symptoms clinically, typically fatigue and dyspnea on exertion.
2. Reticulocyte haemoglobin content (CHr)- It counts the quantity of the iron availability in the bone marow that can be utilized by reticulocyte for synthesis of hemoglobin. A CHr of less than 28 pg is a reliable predictor for iron deficiency anemia[11]

Statistical analysis: Quantitative data is presented with the help of Mean and Standard deviation. Qualitative data is presented with the help of frequency and percentage table. Association among the study groups is assessed with the help of Fisher test, student 't' test and Chi-Square test. 'p' value less than 0.05 is taken as significant.

Pearson's chi-squared test

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

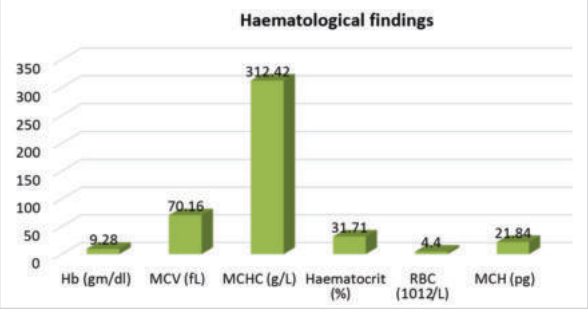
Where X2 = Pearson's cumulative test statistic.
Oi = an observed frequency;
Ei = an expected frequency, asserted by the null hypothesis;
n = the number of cells in the table.

Appropriate statistical software, including but not restricted to MS Excel, SPSS ver. 20 used for statistical analysis.

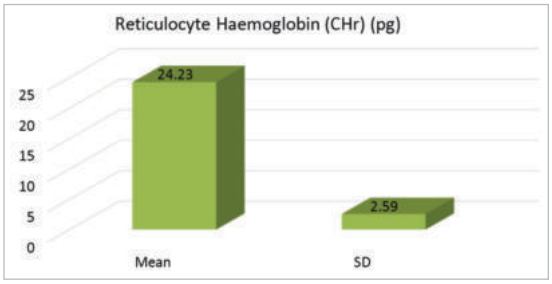
RESULTS –

A hospital based prospective study was conducted with 96 patients to evaluate the clinical significance of reticulocyte haemoglobin content in the diagnosis of iron deficiency anemia.

Haematological findings of patients -The mean Haemoglobin (Hb) and Mean Corpuscular Volume (MCV) values of patients were 9.28 ± 0.99gm/dl and 70.16 ± 5.54fL respectively while the Mean Corpuscular Haemoglobin Concentration (MCHC) and Haematocrit values of patients were 312.42 ± 35.37g/L and 31.71 ± 1.92% respectively. The mean RBC and Mean Corpuscular haemoglobin (MCH) values of patients were 4.40 ± 0.3810^{12}/L and 21.84 ± 0.79pg respectively.



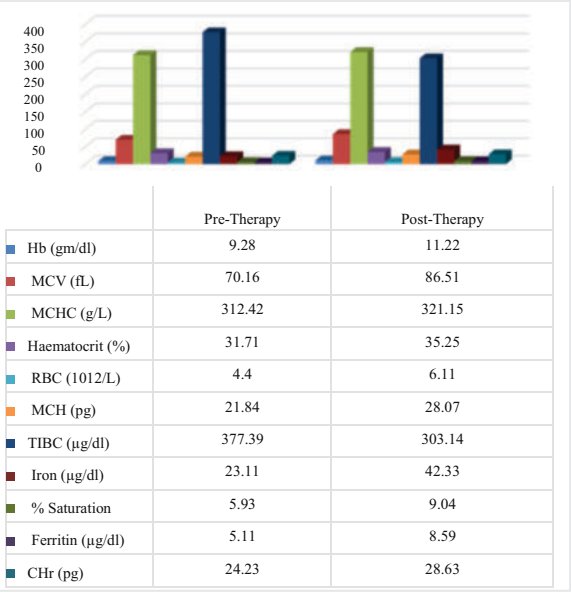
Reticulocyte Haemoglobin (CHr) values of patients - The mean Reticulocyte Haemoglobin (CHr) values of patients was 24.23 ± 2.59pg.



Comparison of Parameters of patients (Pre and Post Iron Supplementation Therapy)

The mean Hb (9.28±0.99gm/dl vs. 11.22±0.97gm/dl), MCV (70.16±5.54fL vs. 86.51±5.59fL), Hematocrit (31.71±1.92% vs. 35.25±2.03%), RBC (4.40±0.38 10^{12}/L vs. 6.11±0.42 10^{12}/L), MCH (21.84±0.79pg vs. 28.07±0.97pg), Iron (23.11±3.38µg/dl vs. 42.33±3.70µg/dl), %Saturation (5.93±2.06 vs. 9.04 ± 2.07), Ferritin (5.11±2.05µg/dl vs. 8.59 ± 2.04µg/dl) and Reticulocyte Haemoglobin (CHr) values (24.23±2.59pg vs. 28.63±2.63pg) were significantly higher while the mean TIBC (377.39±7.85µg/dl vs. 303.14 ± 7.60µg/dl) was significantly lower in patients after iron supplementation therapy as per Student t-test (**p<0.05**). The MCHC values (312.42 ± 35.37g/L vs. 321.15 ± 35.40g/L; p>0.05) was comparable in patients after iron supplementation therapy as per Student t-test (p>0.05).

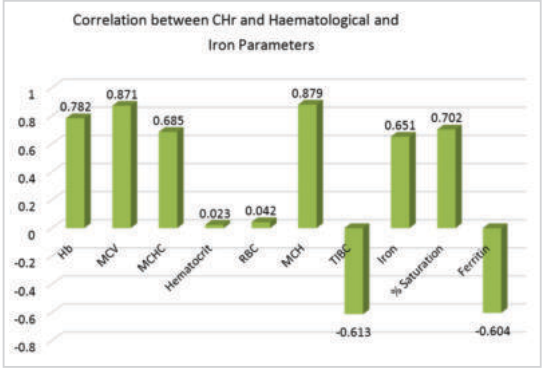
Comparison of Parameters of patients (Pre and Post Iron Supplementation Therapy)



	Pre-Therapy	Post-Therapy
Hb (gm/dl)	9.28	11.22
MCV (fL)	70.16	86.51
MCHC (g/L)	312.42	321.15
Haematocrit (%)	31.71	35.25
RBC (1012/L)	4.4	6.11
MCH (pg)	21.84	28.07
TIBC (µg/dl)	377.39	303.14
Iron (µg/dl)	23.11	42.33
% Saturation	5.93	9.04
Ferritin (µg/dl)	5.11	8.59
CHr (pg)	24.23	28.63

Correlation between Reticulocyte Haemoglobin (CHr) and Haematological and Iron Parameters

Reticulocyte Haemoglobin (CHr)showed a significant positive correlation with haemoglobin (r=0.782; **p<0.05**), MCV (r=0.871; **p<0.05**), MCHC (r=0.685; **p<0.05**), MCH (r=0.879; **p<0.05**), serum iron (r=0.651; **p<0.05**), and % Saturation (r=0.702; **p<0.05**). CHr showed significant negative correlation with TIBC (r=-0.613; **p<0.05**) and Ferritin (r=-0.604; **p<0.05**).



The following results were noted-

1. Majority of the patients (35.5%) were in the age group of 31-40 years The mean age of the patients was 38.55 ± 13.46 years.
2. Female patients constituted 63.5% of the study population.
3. The mean Haemoglobin (Hb) and Mean Corpuscular Volume (MCV) values of patients were 9.28 ± 0.99gm/dl and 70.16 ± 5.54fL respectively while the Mean Corpuscular Haemoglobin Concentration (MCHC) and Haematocrit values of patients were 312.42 ± 35.37g/L and 31.71 ± 1.92% respectively. The mean RBC and Mean Corpuscular haemoglobin (MCH) values of patients were 4.40 ± 0.38 10^{12}/L and 21.84 ± 0.79pg respectively.
4. The mean Total Iron-binding capacity (TIBC) and Iron values of patients were 377.39 ± 7.85µg/dl and 23.11 ± 3.38µg/dl respectively while the %Saturation and Ferritin values of patients were 5.93 ± 2.06 and 5.11 ± 2.05µg/dl respectively.
5. The mean Reticulocyte Haemoglobin (CHr) values of patients was 24.23 ± 2.59pg.
6. The mean Hb and MCV values of patients after iron supplementation therapy were 11.22 ± 0.97gm/dl and 86.51 ± 5.59fL respectively while the MCHC and Haematocrit values of patients were 321.15 ± 35.40g/L and 35.25 ± 2.03% respectively. The mean RBC and MCH values of patients were 6.11 ± 0.4210^{12}/L and 28.07 ± 0.97pg respectively.
7. The mean TIBC and Iron values of patients after iron supplementation therapy were 303.14 ± 7.60µg/dl and 42.33 ± 3.70µg/dl respectively while the %Saturation and Ferritin values of patients were 9.04 ± 2.07 and 8.59 ± 2.04µg/dl respectively.
8. The mean Reticulocyte Haemoglobin (CHr) values of patients after iron supplementation therapy were 28.63 ± 2.63pg.
9. The mean Hb (9.28±0.99gm/dl vs. 11.22±0.97gm/dl), MCV (70.16±5.54fL vs. 86.51±5.59fL), Haematocrit (31.71±1.92% vs. 35.25±2.03%), RBC (4.40±0.38 10^{12}/L vs. 6.11±0.42 10^{12}/L), MCH (21.84±0.79pg vs. 28.07±0.97pg), Iron (23.11±3.38µg/dl vs. 42.33±3.70µg/dl), %Saturation (5.93±2.06 vs. 9.04 ± 2.07), Ferritin (5.11±2.05µg/dl vs. 8.59 ± 2.04µg/dl) and Reticulocyte Haemoglobin (CHr) values (24.23±2.59pg vs. 28.63±2.63pg) were significantly higher while the mean TIBC (377.39±7.85µg/dl vs. 303.14 ± 7.60µg/dl) was significantly lower in patients after iron supplementation therapy as per Student t-test (**p<0.05**). The MCHC values (312.42 ± 35.37g/L vs. 321.15 ± 35.40g/L; p>0.05) was comparable in patients after iron supplementation therapy as per Student t-test (p>0.05).
10. Reticulocyte Haemoglobin (CHr) showed a significant positive correlation with haemoglobin (r=0.782; **p<0.05**), MCV (r=0.871; **p<0.05**), MCHC (r=0.685; **p<0.05**), MCH (r=0.879; **p<0.05**), serum iron (r=0.651; **p<0.05**), and % Saturation (r=0.702; **p<0.05**). CHr showed significant negative correlation with TIBC (r=-0.613; **p<0.05**) and Ferritin (r=-0.604; **p<0.05**).
11. Receiver operating characteristic (ROC) analysis showed that the CHrcut-off of 27.2 pg showed 85.7% sensitivity, 82.2% specificity, 60.8% PPV, 93.4% NPV, and 82.4% accuracy.

DISCUSSION-

Anemia is foremost health problem worldwide.Several biochemical markers are being used for the diagnosis of IDA. Although, there might be few difficulties in the measurement of these conventional parameters. E.g., ferritin acts as an acute phase reactant, which limits its diagnostic accuracy significantly. The serum ferritin level is often raised in condition such as acute/chronic inflammation, cancer, infection, alcohol use and liver disease. Serum iron levels also, reduced with inflammation, infection and malignancy and rises in liver disease.[3]. CHr provides an indirect measure of the functional iron available for new erythrocyte production.

Karagulle M et al[11] studied the clinical significance of reticulocyte haemoglobin content (CHr) in the diagnosis of IDA found no statistically significant difference between the 2 groups in terms of red blood cell (RBC) count and age. Values of Haemoglobin, MCV, MCH, MCHC, transferrin saturation and serum iron, were significantly lesser in female patients with Iron Deficiency anemia in comparison to female patient with iron-deficiency.

Karagulle M et al[11] study found CHr was 24.95±3.92 pg in the IDA group and 29.93±2.96 pg in the iron deficiency group, the 2 groups shows statistically significant difference in respect to Chr.

Mast AE et al[8] study found average CHr was 30.8 6 ±0.90 pg with a range of 28.8 to 32.9 pg. No significant variance amongst female and male values was present. To evaluate its efficacy in predicting iron stores, present in bone marrow.

Karlsson T[12] study observed mean values of Hb, CHr, MCV, MCH, transferrin saturation ,iron, and ferritin were significantly lesser in the IDA group compared to ACD group. In IDA group TIBC and Soluble transferrin receptor were significantly higher in the IDA group, although no difference found for CRP.

Chaipokam J et al[16] prospective study evaluating the use of CHr with microcytic red cells for diagnosis of IDA found mean CHr was 27.3 pg. The CHr values of patients in the thalassemia trait and AI groups were significantly less compared to control group, but significantly greater than those in the groups with IDA or thalassemia disease .

In our study we observed that the mean Reticulocyte Haemoglobin (CHr) values of patients was 24.23 ± 2.59pg. This is consistent with the studies of Karagulle M et al[12], Mast AE et al[8], Karlsson T[12] and Chaipokam J et al[16].

Karagulle M et al[11] study found Transferrin and TIBC were significantly greater in female patients with IDA in comparison of female patients with iron deficiency.

It was observed in the present study that the mean Total Iron-binding capacity (TIBC) and Iron values of patients were 377.39 ± 7.85µg/dl and 23.11 ± 3.38µg/dl respectively while the Saturation and Ferritin levels of patients were 5.93 ± 2.06 and 5.11 ± 2.05µg/dl respectively. This is corresponding with study of Karagulle M et al[11].

In the present study, the mean Hb and MCV values of patients after iron supplementation therapy were 11.22 ± 0.97gm/dl and 86.51 ± 5.59fL respectively while the MCHC and Hematocrit values of patients were 321.15 ± 35.40g/L and 35.25 ± 2.03% respectively. The mean RBC and MCH values of patients were 6.11 ± 0.4210^{12}/L and 28.07 ± 0.97pg respectively.

In our study, the mean TIBC and Iron values of patients after iron supplementation therapy were 303.14 ± 7.60µg/dl and 42.33 ± 3.70µg/dl respectively while the %Saturation and Ferritin values of patients were 9.04 ± 2.07 and 8.59 ± 2.04µg/dl respectively. The mean Reticulocyte Haemoglobin (CHr) values of patients after iron supplementation therapy was 28.63 ± 2.63pg.

In our study we observed that that Reticulocyte Haemoglobin (CHr)revealed a significant positive correlation with haemoglobin (r=0.782; **p<0.05**), MCV (r=0.871; **p<0.05**), MCHC (r=0.685; **p<0.05**), MCH (r=0.879; **p<0.05**), serum iron (r=0.651; **p<0.05**), and % Saturation (r=0.702; **p<0.05**). CHr showed significant negative correlation with TIBC (r=-0.613; **p<0.05**) and Ferritin (r=-0.604; **p<0.05**). Lima J et al[13], Chaipokam J et al[16], Karagulle M et al[11] and Mehta S et al[14] noted similar observations in their studies.

Karagulle M et al[11] study revealed a significant positive correlation of CHr with haemoglobin (r=0.775), MCV (r=0.868), MCH (r=0.883), MCHC (r=0.685), serum iron (r=0.648), and TS (r=0.764) and transferrin (r=-0.599) and TIBC (r=-0.613) significant negative correlation.

Mehta S et al[14] hospital based study evaluating reticulocyte haemoglobin (CHr) in relation with serum ferritin revealed significant difference in means of CHr (Group A 17.84 ± 2.39 vs. Group B 25.08 ± 4.42; P< 0.0001) and serum ferritin (Group A 8.68 ± 2.80 vs. Group B 15.61 ± 4.68; P < 0.0001). A significant positive correlation of ferritin with CHr in total patients (r = 0.7860, p 0.0000), Group A (r = 0.7089, p 0.00) and Group B (r = 0.4675, p < 0.05) patients. CHr was the only significant predictor of bone marrow iron stores (at P < 0.05).

Okam MM et al[15] study reported that Haemoglobin increase of <1.0 g/dL was found at day 14 most accurately predicted satisfactory. overall haemoglobin response to oral iron on day 42/56 (specificity 79.3% sensitivity 90.1%; PPV 92.9% and NPV 72.7%).

CONCLUSION-

CHr is a useful parameter that can be confidently used in the diagnosis of Iron deficiency anemia (IDA) and a CHr cut-off value predicts IDA. Ferric carboxymaltose was usually well tolerated and had a good impact in the management of iron-deficiency anemia. After iron

replacement therapy CHr values increased correlated with haemoglobin and iron level. We cannot replace iron study but CHr can be used for early diagnosis of Iron deficiency anemia and in management of iron deficiency.

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