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Original Research Article

Utility of reticulocyte haemoglobin content (CHr) in the diagnosis of iron deficiency anaemia in adult females

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ABSTRACT

Background: Iron deficiency anaemia is the most common nutritional deficiency to affect people of all age groups. It is frequently detected only in the late stages of the disease by haemoglobin estimates, peripheral smear and iron investigations. Reticulocyte haemoglobin content [CHr] is the newer parameter detected by most haematology analyzers, which is useful in detecting iron deficiency anaemia in the early stages, even before the development of symptoms of anaemia.
Context: Role of CHr in the diagnosis of iron deficiency anaemia. Aim – To evaluate the role of reticulocyte hemoglobin content in the diagnosis of iron deficiency anaemia in adult females. Settings and Design: Cross-sectional study at tertiary care hospital.
Methods and Materials: A 2year observational cross-sectional study was conducted at a tertiary care hospital, after Institutional Ethics Committee approval. Total 76 cases were selected in the study after informed consent. 48 were taken as test samples with hemoglobin level less than 10gm% and 28 were taken as controls with hemoglobin level more than 10gm%. EDTA samples were collected, and their complete blood count (CBC) was analyzed on a 5-part haematology analyzer, followed by analysis of the same sample on the retic mode to determine reticulocyte parameters. The mean, standard deviation and p value were used to evaluate the data**.**
Results: Out of the total 76 patients, 48 were controls and 28 were test cases. Mean Hb in the test group was 7.27 gm% whereas mean Hb in the control group was 12.8gm%. As per the CHr cut-off value obtained by the ROC curve, the mean value for CHr was 22.12 pg in the test group and 27.3pg in the control group. Using the t-test, CHr had a strong positive correlation with the RBC indices.
Discussion: The correlation of the CHr with haematological indicators used to detect iron deficiency anaemia was analyzed in the current study. CHr values in the test group (Hb 10gm%) were significantly lower than in the control group (p 0.05). Significant positive associations were found between CHr and several RBC parameters.

1. Introduction

Iron deficiency anaemia affects roughly 2 billion individuals worldwide, making it the most prevalent nutritional deficiency. If left untreated, it can cause developmental delays in the young and other systemic effects. It is usually diagnosed only when patients present in the late stage by Haemoglobin estimation, peripheral smear examination and iron studies. Fortunately, reticulocyte haemoglobin content [CHr] is a recent parameter identified by most haematology analyzers that is beneficial in diagnosing early iron deficiency anaemia since reticulocytes have a limited life span in the peripheral blood of 1-2 days before complete maturation. When used in conjunction with haemoglobin and red cell parameters available on the analyser, CHr

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can pick up early iron deficiency anaemia. It is a cheaper option than doing iron studies in all cases where iron deficiency anaemia is suspected, especially in early cases. As a result, it can be used to screen for iron deficiency anaemia in conjunction with the Complete blood count [CBC]- especially the Haemoglobin and red cell parameter values.[1]

Anaemia, which affects pregnant women as well as non-pregnant women, is one of the most prevalent and unresolvable health issues. During the latter trimester of pregnancy, pregnant women are more at risk. In addition to being a risk factor for neonatal iron insufficiency, anaemia significantly contributes to maternal mortality and morbidity. Anaemia.[2,3] It is critical to correctly identify iron deficiency in pregnant women as early as possible because it may lead to preterm delivery, low birth weight, developmental defects, behavioural disturbances and perinatal complications if untreated.

Iron deficiency is detected using a range of peripheral blood tests, including ferritin, transferrin saturation, serum iron, and others. Prussian blue staining on a bone marrow biopsy is now the most accurate approach. However, this is time-consuming, costly, and painful. As a result, it is unfit for routine usage.[4] Other markers, such as serum ferritin and serum transferrin receptors, have limitations in terms of sensitivity and specificity.[5]

According to research, Reticulocyte Haemoglobin Content might be a reliable predictor of Iron Deficiency Anaemia.[6,7] Reticulocytes are generated in the bone marrow, after which they circulate for a day or two before developing into mature red blood cells[7]. Because reticulocytes have a shorter life cycle, Reticulocyte Haemoglobin Content (CHr) is a superior biomarker because it reveals the availability of iron for erythropoiesis. In contrast to ferritin, CHr has a high specificity since it is unaffected by inflammation. In comparison to a traumatic bone marrow biopsy, CHr is less expensive and less intrusive because just a few millilitres of blood are required to get CHr data. Hence CHr maybe a suitable alternative to bone marrow examination.[8] Various studies have shown the normal range of CHr in adults varies from 26-32.9pg.[1,9–11]

2. Primary Objective

To find out the role of CHr for diagnosing of iron deficiency anaemia in adult females.

3. Materials and Methods

3.1. Study design

This was a cross-sectional observational study undertaken after Institutional Ethics Committee approval was obtained.

3.2. Subjects

Total 76 female patients attending our tertiary care hospital were selected in the study after informed consent. Out of 76, 48 were taken as test samples with haemoglobin level less than 10gm% and 28 were taken as controls with haemoglobin level more than 10gm%. All participants in this study provided informed consent. Study subjects having abnormal bleeding or recent iron supplementation were excluded from the study.

3.3. Analytical methods

The EDTA samples of all the patients were taken and Complete blood count (CBC) was analysed on a 5-part haematology analyser (ADVIA 2120), followed by analysing the same sample on the retic mode to analyse reticulocyte parameters including reticulocyte count and reticulocyte haemoglobin content. Samples were run within 2 hours of collection. The iron study data was recorded where available and recommended wherever necessary.

4. Results

Data obtained was analysed using appropriate software. For hematologic variables, the mean and standard deviation (S.D.) were calculated. The P value evaluated the relationship between variables. $P < 0.05$ was considered significant.

Total 76 patients were analysed in the study out of which 48 were test subjects and 28 were controls. Their ages ranged between 18 to 75 years and mean age was 36.2 years and 27.5 years amongst test and control samples respectively. Mean Hb in the test group was 7.27 gm% whereas mean Hb in the control group with Hb >12gm% was 12.8gm%. The mean value for CHr was 22.12 pg in the test group and 27.3pg in the control group (Tables 1 and 2).

The t-test for independent samples was used to compare CHr to RBC indices (MCV, MCH, MCHC). CHr and RBC indices had a strong positive correlation (p value < 0.05).

To identify the optimal cut of value of CHr, a receiver operating characteristic (ROC) curve graph was plotted, and hence using a CHr cut off point of 25.4pg, the sensitivity was 60.7%, specificity was 83.3% and accuracy of 75% for the diagnosis of anaemia.(Table 3)

The data regarding iron studies of the patients (serum iron, serum ferritin and TIBC) were sparse. Hence, all the coefficients were statistically non-significant.(Figure 1)

5. Discussion

Diagnosis of iron deficiency anaemia there by the conventional iron studies have shown a wide variation due to disease states, pregnancy and other infective or inflammatory factors, which results in falsely high or low levels. Since Ferritin is an acute phase reactant,

Table 1: Controls and test samples.

Controls (Hb > 10 gm%)	28
Test Samples (Hb < 10gm%)	48
Total	76

Table 2: Mean values of different parameters between two groups based on haemoglobin.

Mean	**Test (n = 48)**	**Control (n = 28)**	
	Hb <10 gm%	Hb 10-12 gm% n = 19	Hb >12 gm% n = 9
Age	36.2 years	27.8 years	26.4 years
Hb	7.27 gm%	10.9 gm%	12.8 gm%
Hematocrit	24.59	34.58	39.94
RBC count	3.69	4.71	5.08
MCV	68.34	73.76	79.89
MCH	20.92	23.6	25.9
MCHC	29.17	31.67	32.45
Retic count	1.49	1.48	1.6
CHr	22.12	25.01	27.3

Table 3: Comparison of different parameters between two groups based on haemoglobin.

Parameters	**Haaemoglobin category**										**P-value***
	Hb <= 10 gm%					Hb > 10 gm%					
	n	Mean	SD	Minimum	Maximum	n	Mean	SD	Minimum	Maximum	
Age in years	48	36.25	15.08	18.00	75.00	28	27.54	9.81	21.00	69.00	**0.008**
HCT	48	24.59	6.35	8.00	35.20	28	36.48	4.24	30.90	51.00	**< 0.0001**
RBC	48	3.69	1.14	1.07	5.69	28	4.83	0.83	3.76	7.80	**< 0.0001**
MCV	48	68.34	9.78	51.70	90.80	28	76.53	8.64	58.00	92.20	**< 0.0001**
MCH	48	20.92	4.43	12.10	30.20	28	24.60	3.77	17.60	30.80	**< 0.0001**
MCHC	48	29.17	4.67	12.70	35.20	28	32.07	1.94	28.50	35.80	**0.003**
RDW	48	20.57	4.51	14.50	33.70	28	15.79	2.48	12.50	21.70	**< 0.0001**
CHr	48	22.12	5.82	2.07	44.30	28	25.97	3.93	18.70	33.30	**0.003**

*Obtained using t-test for independent samples; Bold p-values indicate statistical significance

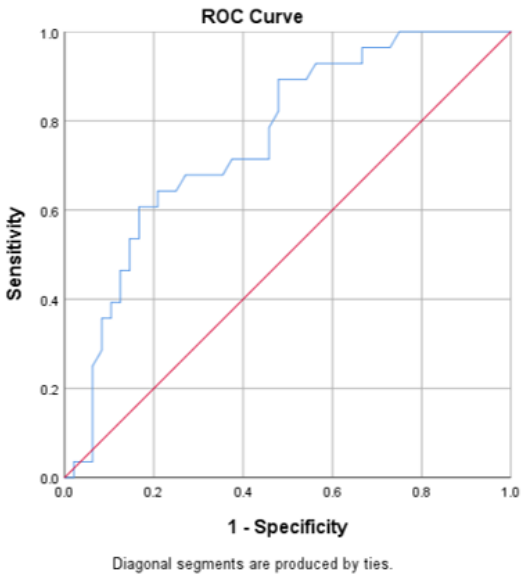

Figure 1: Receiver operating characteristic curve forCHr for patients grouped according to Hb.

its diagnostic accuracy is restricted. CHr (reticulocyte haemoglobin content) is a parameter not affected by any confounding factors. It represents the iron content of the reticulocyte, which is a direct representation of the bone marrow's iron status and a measure of functional iron accessible for fresh RBC formation.

In our study, we studied the correlation of CHr with routine haematological parameters used to diagnose iron deficiency anaemia in test and control groups. The results yielded an acceptable correlation. In test group (Hb < 10gm%), significantly low CHr values were observed as compared to controls ($p < 0.05$). Significant positive correlations were observed between CHr and various RBC indices.

According to Poffenroth et al[1] the reticulocyte haemoglobin equivalent at a cut-off of < 26pg may indicate the need for iron studies, with values over the cut-off functioning as a negative predictor of iron shortage. A study done at Department of Laboratory Medicine, Harvard Medical School, Ret He (reticulocyte haemoglobin equivalent) was shown to be a reliable measure of cellular

haemoglobin content and can be used to detect iron deficiency disorders.[10] As per the study done by Cai et al,[4] 140 adults were divided into three groups – iron deficiency anaemia (IDA) group, non-iron deficiency anaemia (NIDA) group and a control group based on the haemoglobin cut-offs. The IDA group had considerably reduced levels of reticulocyte haemoglobin, MCV, MCH, and haemoglobin content. This is in concordance with our study. Functional iron deficit was defined as CHr < 28pg in a study by Thomas et al[12]

The current study showed positive correlation between CHr and the red blood cell indices. According to a study done by Karagulle M et al.[3] CHr demonstrated the highest association with haemoglobin, MCV, and MCH and seemed to be reliable in detecting IDA. In a study done by Kariyawasan C.C et al,[13] positive associations were found between CHr as well as biochemical indicators such as serum iron and serum ferritin. Due to non-availability of data on the biochemical parameters in our study, no statistically significant correlation could be established in terms of CHr.

Many investigations have shown that ferritin behaves as an acute phase reactant, which severely restricts its diagnostic accuracy. Many variables influence its value irrespective of iron status. Serum iron levels are also affected by infections, inflammation and malignancy. CHr measurement gives an indirect assessment of the functional iron available for the formation of new RBCs.[14–16]

6. Conclusion

The correlation between CHr and conventional haematological parameters indicate that CHr may be a good and reliable predictor of iron deficiency anaemia. The rapid diagnosis on the EDTA sample during routine blood testing for CBC, on the same haematology analyser and at a reasonable cost is an added advantage.

7. Source of Funding

None.

8. Conflict of Interest

None.

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