Evaluation of Reticulated Haemoglobin (CHr) as a diagnostic parameter in Iron Deficiency Anemia

C. C. Kariyawasan, D. J. U. S. Samarasekara, N. Vithanage, D. M. C. Dissanayake,
S. A. C. D. Ranatunga, and B. L. T. Balasuriy

Abstract — Introduction: Iron deficiency anemia (IDA) is the commonest nutritional deficiency in all parts of the world. In developing countries, the commonest cause is inadequate dietary intake [1]. The red cell indices raise the suspicion of iron deficiency state due to the microcytic, hypochromic changes and the wide red cell distribution width. The iron studies are confirmatory of an iron deficiency state, but are not accurate in the presence of infection, inflammation or malignancy as they are acute phase proteins and are affected in these states making the serum iron studies unreliable under these conditions. The reticulocyte haemoglobin content (CHr) indicates the iron available in the marrow for the production of Hb and are not affected by the above-mentioned situations and therefore has been recommended as a reliable marker of iron status in the body. The value for CHr given in the literature was 25pg [3].

Objectives: General objective was to evaluate the significance of CHr in the diagnosis of IDA. The Specific objectives were to compare CHr with the other conventional iron parameters including serum iron, serum ferritin, TS and TIBC and evaluate any significance of CHr with RBC indices (MCV, MCH and MCHC) and age.

Methodology: A retrospective analytical study performed at the Department of Haematology of a Tertiary care hospital in Sri Lanka. Data was obtained from the patient records of those referred to the Haematology department for management of iron deficiency during the period of 9 months commencing from April 2019 to January 2020. Data from 178 adult patients both males and females (16 to 84 years) diagnosed with IDA (S. Ferritin < 20ng/ml) (Hb < 12g/dl in men and Hb < 11.5g/dl in women) were randomly selected. In pregnant females the S. Ferritin level considered was <30ng/ml and the Hb level was <11g/dl in the first trimester and 10.5g/dl in the 2nd and third trimester [4]. Blood count and CHr was analyzed using Mindray fully automated analyzer BC 6800, Serum iron and TIBC was measured with Mindray BS 480 and Serum ferritin with Advia Centaur Xp. TS was calculated by dividing serum iron by TIBC and multiplying by 100. A data extraction sheet was used to enter the investigations with the results.

Statistical Analysis: Data were double entered and were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistical methods were used to calculate the median and the mean $\pm$ standard deviation of Hb, serum iron, serum ferritin, TIBC, TS, MCV, MCH, MCHC and CHr. Pearson's correlation was used to evaluate the correlation between variables. Coefficient of determination (R

Sq) was used to a statistical measure of how close the data are to the fitted regression line. $P < 0.05$ was considered significant.

Conclusion: Significant positive correlations were observed between the CHr and haematological parameters such as Hb, MCV, MCH, and MCHC and biochemical parameters including serum iron, serum ferritin, and TS (p value < 0.001). Negative correlation was seen between the CHr and TIBC and there was no correlation with age. The mean value of CHr was 22.4 ± 4.16 pg and median was 22.2 pg.

Index Terms — Iron deficiency anemia, Serum iron studies Reticulocyte haemoglobin content (CHr).

I. INTRODUCTION

Iron deficiency anemia (IDA) is the commonest nutritional deficiency in all parts of the world. The reason for the deficiency is multifactorial but in developing countries, the commonest is inadequate dietary intake [1].

The laboratory tests which are done to confirm an iron deficiency state include serum iron, total iron binding capacity (TIBC) transferrin saturation (TS), and serum ferritin (serum iron studies). Of these most specific would be the serum ferritin but being an acute phase protein would be difficult to interpret in the presence of inflammation, infection or malignancy [2]. The serum iron is reduced in states of infection, inflammation and malignancy besides iron deficiency and increase with liver disease. The TS is affected by the diurnal variation of iron. It is recommended to measure C- reactive protein if serum ferritin is normal or high with serum iron or transferrin saturation below their respective reference intervals. Transferrin saturation <16% is poorly specific, as pregnancy, oral contraceptive use, and chronic illness can result in low transferrin saturation without iron deficiency.

Reticulocytes are the immediate precursor cells of mature erythrocytes to be released by the marrow into the peripheral blood. They circulate in the blood for 1-2 days and transform into mature erythrocytes. The reticulocyte haemoglobin content (CHr) indicates the iron available in the marrow for the production of Hb. CHr has been proposed as a marker of iron status in the body as it is not affected by any of the conditions affecting all the parameters in an iron profile. Many studies have shown that blood CHr measurement is useful in the diagnosis of IDA and that it could be used in the monitoring of iron treatment. This would be a very useful test to be used in low income countries such as Sri Lanka due to its low cost in contrast to the iron profile. The requirement of just 2CC of EDTA blood in contrast to 4CC of blood taken into 2 containers too makes it a more acceptable test. The cut off value of the CHr has been given as 25pg [3].

Published on July 1, 2020.

C. C. Kariyawasan, Sri Jayewardanepura General Hospital, Sri Lanka.
(corresponding e-mail: chitrak64@yahoo.com).

D. J. U. S. Samarasekara, Sri Jayewardanepura General Hospital, Sri Lanka.

N. Vithanage, Sri Jayewardanepura General Hospital, Sri Lanka.

D. M. C. Dissanayake, Sri Jayewardanepura General Hospital, Sri Lanka.

S. A. C. D. Ranatunga, Sri Jayewardanepura General Hospital, Sri Lanka.

B. L. T. Balasuriy, Sri Jayewardanepura General Hospital, Sri Lanka.

II. OBJECTIVES

A. General Objective

To evaluate the significance of CHr in the diagnosis of IDA.

B. Specific Objectives

1. To compare CHr with the other conventional iron parameters including serum iron, serum ferritin, TS and TIBC.
2. Evaluate any significance of CHr with RBC indices (MCV, MCH, MCHC) and age.

III. METHODOLOGY

A. Study Design

A retrospective analytical study.

B. Center of the study

Department of Haematology.

C. Sample selection and sample size

The data from patients registered during the period of 9 months commencing from April 2019 to January 2020 were included in the study. 178 adult patients both males and females (16 to 84 years) diagnosed with IDA (S. Ferritin < 20ng/ml) (Hb < 12g/dl in men and Hb < 11.5g/dl in women) were randomly selected from those referred to the Haematology department for management of iron deficiency. In pregnant females the S. Ferritin level considered was <30ng/ml and the Hb level was <11g/dl in the first trimester and 10.5g/dl in the 2nd and third trimester [4]. Patients with a history of haematological disorders (i.e., thalassaemia trait, sickle cell trait, megaloblastic anaemia and malignant conditions) or HIV status were excluded from the study [5].

D. Data Collection

From the patient's records blood count, CHr, Serum iron, TIBC, Serum ferritin and TS (calculated by dividing serum iron by TIBC and multiplying by 100) were obtained. A data extraction sheet was used to enter the investigations with the results. The FBC and CHr were analyzed using Mindray fully automated analyzer BC 6800, Serum iron, and TIBC was measured with Mindray BS 480 and Serum ferritin with Advia Centaur Xp.

E. Statistical Analysis

Data were double entered and were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistical methods were used to calculate the median and the mean $\pm$ standard deviation of Hb, serum iron, serum ferritin, TIBC, TS, MCV, MCH, MCHC and CHr. Pearson's correlation was used to evaluate the correlation between variables along with the r value which if positive indicates a positive correlation and vice versa. Coefficient of determination (R Sq) was used to a statistical measure of how close the data are to the fitted regression line. $P < 0.05$ will be accepted as significant.

IV. RESULTS

Of the total of 178 patients' data analyzed, 171 were female. Only 7 were males. Age range was between 16 to 84 years and mean age was 41.1 years. Hb range was 4.1–11.5 g/dl with the mean of 8.5 ± 1.76 g/dl. Mean values for serum iron, serum ferritin, TIBC and TS were 5.82 ± 5.57 μ mol/L, 9.47 ± 5.73 ng/ml, 73.7 ± 15.66 μ mol/L, $8.37 \pm 8.31\%$ respectively. The mean value of CHr was 22.4 ± 4.16 pg and median was 22.2 pg (Table 1).

TABLE 1 DESCRIPTIVE STATISTICS FOR STUDY POPULATION

	Number	Mean	Median	Mode	Std. Deviation	Min.	Max.
Hb	178	8.50	8.60	9.5	1.76	4.1	11.5
Iron	178	5.82	4.00	4.0	5.57	1.0	37.0
TIBC	178	73.70	73.00	76.0	15.66	44.0	131.0
Ferritin	178	9.47	8.05	19.0	5.73	0.7	19.8
CHr	178	22.4	22.20	22.0	4.16	14.8	33.7
MCV	178	74.40	74.00	69.0	11.56	51.2	100.0
MCH	178	22.5	22.6	1.7	4.52	13.3	33.7
MCHC	178	30.13	30.25	29.6	1.94	24.9	35.0
TS	178	8.37	5.86	2.5	8.31	1.1	17.0

To evaluate the significance of CHr in the diagnosis of IDA, Pearson's correlation was done between CHr and serum ferritin and Hb (Table 2). There was a significant positive correlation between CHr and serum ferritin ($r=0.578$, p value <0.001) with the R Sq of 0.2 and showing a moderate linear relationship (Fig 1). A significant positive correlation between CHr and Hb ($r=0.592$, p value <0.001) showed a moderate linear relationship with R Sq of 0.329.

Table 2 further reveals the comparison of CHr with the other conventional iron parameters including serum iron and TS.

TABLE 1 CORRELATION BETWEEN CHr AND MEAN RESULTS OF AGE, HAEMATOLOGICAL PARAMETERS AND BIOCHEMICAL PARAMETERS

	Correlation (r value)	R Squared value	p- value
Age	0.023	-	0.760
Hb	0.592	0.329	<0.001
MCV	0.724	0.512	<0.001
MCH	0.760	0.473	<0.001
MCHC	0.612	0.302	<0.001
S. Iron	0.209	0.191	<0.001
S. Ferritin	0.578	0.200	<0.001
TIBC	-0.224	0.042	<0.001
TS	0.266	0.253	<0.001

A positive correlation was observed between CHr and serum iron ($r=0.209$, p value <0.001), and CHr and TS ($r=0.266$, p value <0.001). R Sq values showed a low linear relationship between CHr and serum iron (R Sq of 0.191) and between CHr and TS (R Sq of 0.253).

There was a significant negative correlation between CHr and TIBC ($r=-0.224$, p value <0.001) which also revealed a low linear relationship (R Sq of 0.042).

Comparison of CHr with RBC indices (MCV, MCH, MCHC) was done by Pearson correlation. (Table 2). There was a significant positive correlation between CHr and RBC indices (p value <0.001 and positive r values).

R squared analysis (Fig. 1) showed a moderate linear relationship between CHr and MCV, CHr and MCH and MCHC. There was no statistically significant correlation between CHr and age (p value =0.76).

Fig. 1. R squared analysis of CHr and other haematological and biochemical variables.

V. CONCLUSION

Comparison of this CHr test with the conventional hematological and biochemical tests commonly used to diagnose iron deficiency anemia, yielded an acceptable correlation. Significant positive correlations were observed between the CHr and haematological parameters such as Hb, MCV, MCH, and MCHC and biochemical parameters including serum iron, serum ferritin, and transferrin saturation. A significantly negative correlation was seen between the CHr values and TIBC.

We conclude CHr can be a good predictor of IDA as evidenced by the correlations between the CHr and conventional haematological and biochemical parameters of iron deficiency anemia.

The CHr testing would enable a rapid diagnosis of IDA with the routine FBC analysis, on the same Haematology Analyzer at a little incremental cost and time. It is not affected by any infective and inflammatory changes unlike the conventional iron studies allowing a clear diagnosis of the iron status even in disease states.

VI. DISCUSSION

Due to the wide variation of iron studies resulting in falsely high or low levels due to the influence on these parameters by disease states, pregnancy and intake of OCP makes management of patients with iron deficiency anemia based on such results difficult. CHr is a very useful parameter which is not controlled by such compounding factors. It is a measurement indicating the iron content in the reticulocyte which is a direct reflection of the iron available in the bone marrow for the synthesis of haemoglobin [6].

The limitations of CHr test is the fact it is calculated using mean cellular volume (MCV) and is calculated based on light scatter information of reticulocytes by the BC 6800 Mindray Haematology Analyzer. It is usually low in the presence of microcytic red cells and may be seen in cases of thalassemia and hemoglobinopathies which cause microcytic anemia. The CHr is also elevated in the cases of megaloblastic anemia because of the high mean cellular volume associated with megaloblastosis. Therefore, it is important that CHr values be interpreted in the patient's overall erythrocyte physiology, including knowledge of recent blood transfusions, iron therapy, vitamin B12 or folate deficiency, and the results of hemoglobin analysis [9].

The cost for a full iron profile is approximately LKR 1600/= whereas the confirmation of anemia and the CHr costs only approximately LKR 600/=. The serum iron studies consisting of S. iron, TIBC, TS and S. Ferritin requires blood (2 cc each) to be collected to two tubes respectively due to the different methods of analysis, whereas for CHr and the FBC a single sample (2 cc) is sufficient. All these confirm CHr as an easy and cost-effective investigation to detect iron deficiency anemia.

According to Brugnara et al, CHr is the strongest predictor of iron deficiency and iron deficiency anemia in children. And they say, serum ferritin test has little or no diagnostic value in children. [7]. Syed et al have found that the CHr is considered to be an initial and reliable indicator

of iron deficiency anemia, and unlike many other iron markers, is not affected by inflammation [8].

In a study performed by Mast et al., it was reported that CHr of < 28 pg had an optimal sensitivity (74%) and specificity (73%) for diagnosis of iron deficiency, using Prussian blue staining of the bone marrow aspirate to define iron deficiency [6]. In our study, the cut off value for CHr was 22.4 ± 4.16pg.

REFERENCES

- [1] WHO Report, World Prevalence of Anemia 1993–2005. *WHO Global Database on Anemia*, World Health Organization, Geneva, Switzerland, 2008, <http://www.WHO.int/hinari/en/>.
- [2] E Urrechaga, L Borque, J F Escanero, "Erythrocyte and Reticulocyte Indices on the LH 750 as Potential Markers of Functional Iron Deficiency", *Anemia*, Article ID 625919, 7 pages. 2010.
- [3] M B Agarwal, S Pai, "Reticulocyte Hemoglobin Content (CHr): The Gold Standard for diagnosing iron deficiency", *Journal of the Association of Physicians of India*, 16: 11-12, 2017.
- [4] M Auerbach, M K Georgieff, "Guidelines for iron deficiency in pregnancy: hope abounds", *British Journal of Haematology*, 188: 6: (814-816), 2019.
- [5] G Chathuranga, T Balasuriya, R Perera, "Anaemia among Female Undergraduates Residing in the Hostels of University of Sri Jayewardenepura, Sri Lanka", *Anemia*, Article ID 526308, 5 pages, 2014.
- [6] A E Mast, M A Blinder, Q Lu, S Flax, D J Dietzen, "Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency", *Blood*: 99:1489-1491, 2002.
- [7] C Brugnara, D Zurakowski, J Dicanzio, T Boyd, O Platt, "Reticulocyte haemoglobin content to diagnose iron deficiency in children". *Journal of American Medical Association*, 281:2225-2230, 1999.
- [8] S Syed, S Kugathasan, A Kumar et al, "Use of Reticulocyte Hemoglobin Content in the Assessment of Iron Deficiency in Children with Inflammatory Bowel Disease", *Journal of Pediatric Gastroenterol Nutrition*. 2017; 64(5): 713–720.
- [9] A E Mast, M A Blinder, D J Dietzen, Reticulocyte hemoglobin content, *American Journal of Hematology*, 2008: 83:307–310.

Dr. (Ms.) C C Kariyawasan MBBS, Diploma in Pathology, MD in Haematology
Dr. Chitranga Kariyawasan was born in 1964, June 6th, Colombo, Sri Lanka. She received undergraduate medical training at the North Colombo Medical College, Sri Lanka and obtained 2nd class honours at the final MBBS. Post graduate qualifications include, Diploma in Pathology and MD in Haematology from the Post Graduate Institute of Medicine (PGIM), University of Colombo, Sri Lanka in the year of 2002 and 2004

respectively.

Currently she is the Consultant Haematologist attached to the Sri Jayewardenepura general Hospital (SJGH) and Post Graduate Training Institute. Dr. Chitranga has held this post from 2009. Prior to joining SJGH, she was a senior lecturer in Pathology at the faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka for 12 years. She was president of the Sri Lanka College of Haematologists in the year 2015 and a resource person at the Highlights of American Society of Haematology (ASH) in Asia held in Brisbane 2016, on the topic of thrombosis.

Dr. Chitranga has publications in refereed journals on the topics of Multiple Myeloma, Hypereosinophilic syndrome, Immune thrombocytopenic purpura, advanced FBC parameters such as immature platelet fraction and rare findings on flowcytometry analysis. Dr. Chitranga is currently an examiner for the Diploma and MD in Clinical Haematology examinations conducted by the PGIM. She is a life member of the Sri Lanka College of Haematologists, College of Pathologists of Sri Lanka and the Sri Lanka Medical Association (SLMA). Other interests include music and theatre having performed in more than 35 stage productions, 10 television productions and for the first time in 2018, presented publicly her own set of plays written and directed by her

Dr. (Mrs) D. J. U. S. Samarasekara, MBBS

Dr. Samarasekara was born in Mirigama, Sri Lanka on 29th June 1975. She was a graduate from University of Colombo, Sri Lanka where she obtained her MBBS in 2003.

She completed internship as a Medical Officer in Base Hospital, Negombo, Sri Lanka in 2005. And later, she joined Sri Jayewardenepura General hospital as a preliminary grade Medical Officer in 2009 and worked in Nephrology and Kidney transplant Unit for 2 years. Since 2011, attached to the Department of Haematology in Sri Jayewardenepura General hospital. Currently, working as a grade 2 Medical Officer with 9 years' experience in Haematology laboratory in Sri Jayewardenepura General hospital.

Dr. (Mrs) N. Vithanage Diploma in Pathology, MD in Chemical Pathology

Dr. Neranjana Vithanage was born in Colombo, Sri Lanka on 25th December 1977. She was a graduate from University of Sri Jayewardenepura, Sri Lanka where she obtained her MBBS in 2004.

She attended University of Colombo, Sri Lanka, for her postgraduate studies and obtained Diploma in Pathology, in 2009 and MD in Chemical Pathology, in 2011. During her overseas training as a Specialty Registrar

in Chemical Pathology, Kings College Hospital NHS Trust, London, United Kingdom, from September 2013 to June 2015 she managed to get through FRCPath Part 1 examination, Royal College of Pathologists, UK in September 2014.

After her return to Sri Lanka, Dr. Neranjana served as the Consultant Chemical Pathologist, to the Ministry of health Sri Lanka at Provincial General Hospital, Badulla, Sri Lanka, and then joined the current workplace, Sri Jayewardenepura General Hospital, Nugegoda, Sri Lanka, serving up to date, as the Consultant Chemical Pathologist.

Dr Vithanage is a council member of College of Chemical Pathologists Sri Lanka.

Mrs. D. M. C. Dissanayake BSc in Medical Laboratory Sciences (Special)

Mrs. D M C Dissanayake was born in Narammala, Sri Lanka on 11th August, 1989. She completed the BSc (Special) degree in Medical Laboratory Sciences at the Faculty of Medical Sciences, University of Sri Jayewardenepura in 2014.

She is a Medical Laboratory Technologist attached to the Department of Haematology of the Sri Jayewardenepura General Hospital, Sri Lanka since 2016. Mrs. Dissanayake has

publications in refereed journals on the topic of Immature Platelet fraction. Mrs. Dissanayake is a registered Medical Laboratory Technologist of the Sri Lanka Medical Council (SLMC) and the Ceylon Medical College Council (CMCC). And she is a member of the College of Medical Laboratory Scientists, Sri Lanka.

Mr. S. A. C. D. Ranatunga B.Sc in Medical Laboratory Sciences (Special), M.Sc in Molecular Pathology (Reading)

Mr. S A C D Ranatunga was born in Colombo, Sri Lanka on 29th November 1990. He completed the B.Sc (Special) in Medical Laboratory Sciences at the Faculty of Medical Sciences, University of Sri Jayewardenepura in 2016 and Certificate course of Practical skills of Molecular Biology and Genetics at Institute of Research & Development. He is studying M.Sc in Molecular Pathology at the Faculty of Medicine, University of Colombo.

He is a Medical Laboratory Technologist attached to the Department of Haematology of the Sri Jayewardenepura General Hospital, Sri Lanka since 2017. Mr. Ranatunga has publications in refereed journals on the topics of subfertility of males, Multiple myeloma, Hairy Cell Leukaemia, aberrant expression of Acute myeloid leukaemias and Sezary syndrome.

Mr. Ranatunga is a registered Medical Laboratory Technologist of the Sri Lanka Medical Council (SLMC) and the Ceylon Medical College Council

(CMCC). And he is a member of the College of Medical Laboratory Scientists, Sri Lanka.

Mr. B. L. T. Balasuriya BSc in Medical Laboratory Sciences (Special)

Mr. B L T Balasuriya was born in Ragama, Sri Lanka on 22nd May 1986. He completed the BSc (Special) degree in Medical Laboratory Sciences with 2nd class upper division at the Faculty of Medical Sciences, University of Sri Jayewardenepura in 2012.

He Started his carrier as a Demonstrator of the Department of Allied Health Sciences in the University of Sri Jayewardenepura. And later, He was appointed as a lecturer attached to the Biomedical Science Degree program of the Colombo branch of Management and Science University, Malaysia. Currently he is a Medical Laboratory Technologist attached to the Department of Haematology of the Sri Jayewardenepura General Hospital, Sri Lanka since 2016.

Mr. Balasuriya has publications in refereed journals on the topics of Anemia, Multiple myeloma, Hairy Cell Leukaemia, aberrant expression of Acute myeloid leukaemias and Sezary syndrome. Mr. Balasuriya is a registered Medical Laboratory Technologist of the Sri Lanka Medical Council (SLMC) and the Ceylon Medical College Council (CMCC). And he is a member of the College of Medical Laboratory Scientists, Sri Lanka.