Reticulocyte Parameters in Microcytic Hypochromic Anaemias Using Beckman Coulter DxH800 – A Study from Kerala, India

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ABSTRACT

BACKGROUND

Microcytic hypochromic anaemia is commonly due to iron deficiency, anaemia of chronic disorder [ACD] and thalassaemic syndromes. Reticulocyte count reflects the erythropoietic activity of bone marrow and is thus useful in both diagnosing anaemias and monitoring bone marrow response to therapy.

METHODS

All samples were selected from routine blood counts, and sent for investigation of anaemia, over a period of two years. These samples were run on the DxH800 (Beckman Coulter). 385 cases were selected for the study. Blood analysis for all these cases had been requested by general practitioners to investigate anaemia. These blood samples had been collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant vacutainers and processed within 2 hours of collection. Determination of red cell and reticulocyte parameters in all blood samples, was performed using the Beckman Coulter 7-part analyser [Unicell DxH 800].

RESULTS

Of the 156 cases of microcytic hypochromic anaemia studied, iron deficiency anaemia (IDA) was present in 91 cases, anaemia of chronic disorder (ACD) in 50 cases, beta thalassemia trait (BTT) in 15 cases. Of the 50 ACD cases, 37 were associated with IDA. The control group comprised of 229 adult medical students (143 women and 103 men) with a median age of 18.84 ± 0.98 years. We also had 4 cases of other haemoglobinopathies, which were microcytic hypochromic, but were not included in our study as the number of cases was too less to be analysed.

CONCLUSIONS

New reticulocyte parameters are useful for evaluation of iron status and diagnosing iron deficiency anaemias. They also are reliable parameters for recognising subsets of anaemic patients thereby improving the management of anaemia.

KEYWORDS

Reticulocyte, Microcytic, Hypochromic, Anaemia, Beckman Coulter

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BACKGROUND

Microcytic hypochromic anaemia is commonly due to iron deficiency, anaemia of chronic disorder [ACD] and thalassaemic syndromes. Of these, ACD is the most common type of anaemia in hospitalised patients. The reticulocyte count reflects the erythropoietic activity of bone marrow and is thus useful in both diagnosing anaemias and monitoring bone marrow response to therapy.

New automated blood cell analysers can provide information about individual cell characteristics, including the haemoglobin content of reticulocytes and mature red blood cells and percentages of microcytic red cells and hypochromic red cells. These new parameters have been used in the diagnosis of iron deficiency anaemia (IDA), β thalassemia (β-thal) carriers and anaemia of chronic disease (ACD). The differentiation between these three conditions is very important as the clinical approach is unique to each particular condition.[1-8]

These reticulocyte parameters include immature reticulocyte fraction (IRF), mean reticulocyte volume (MRV), red blood cell size factor (RSF), low haemoglobin density (LHD) and microcytic anaemia factor (MAF). Immature reticulocyte fraction (IRF) a very early and sensitive index of marrow erythropoietic activity. It is calculated as a ratio of immature reticulocytes to the total number of reticulocytes. The reference range is 0.18 - 0.39 %.[9] Mean reticulocyte volume [MRV] is a measurement of the size of recently produced RBCs within a period of less than 2 days. The reference range is 93.1 - 114.8 fL[9] in adults. The red blood cell size factor [RSF] is another new parameter provided by Beckman Coulter, which relates to volume of erythrocytes and reticulocytes and is calculated as RSF = $\sqrt{\text{MCV}}\text{xMRV}$. The reference range is 91.1 – 106.9 fl [mean 100.9 fl].[10] In a study by Urrechaga[11] it is clear that there is good correlation between RSF and reticulocyte haemoglobin content. Low haemoglobin density (LHD %) is a new parameter provided by Beckman-Coulter derived from the mean cell haemoglobin concentration, using the mathematical sigmoid transformation LHD % = 100 × √ (1 - (1 / (1 + e (1.8 (30 -MCHC)).[9] The reference range is 5.3 – 10.4 %[12]

MAF is the product of haemoglobin (Hb) and mean cellular volume (MCV). MAF = (Hb × MCV) / 100. The reference range is 9.6 - 16.6.[13]

Objectives

To evaluate the utility of reticulocyte parameters in differential diagnosis of IDA from other microcytic hypochromic anaemias such as anaemia of chronic disease (ACD) and beta thalassemia trait (BTT).

METHODS

This was a cross sectional study conducted in Believers Church Medical College Hospital, Thiruvalla, Kerala for a period of 2 years from July 2018 July 2020.

Inclusion Criteria

- Cases include those with Hb < 12.0 g / dL for women and Hb < 13.0 g / dL for men, MCV < 80 fl & MCH < 27 pg.
- Control group comprised of 229 of healthy individuals with no clinical signs or symptoms of disease, acute inflammation / infection and with normal haematologic parameters.

Parameters Collected

- RBC indices: Hb, RBC, MCV, MCH and red blood cell distribution width (RDW).
- Reticulocyte parameters: Reticulocyte %, immature reticulocyte fraction (IRF), mean reticulocyte volume (MRV), red blood cell size factor (RSF), low haemoglobin density (LHD) and microcytic anaemia factor (MAF).

Study Implementation Plan / Logistics

- Blood analysis of all cases for whom investigation of anaemia was requested by general practitioners within the study period and processed by collecting in EDTA anticoagulant vacutainers within 2 hrs of collection on the DxH800 (Beckman coulter).
- Patients were classified according to iron status analysis as:
  i. IDA - Serum iron (Se Fe) levels < 60 g / dL, total iron binding capacity (TIBC) > 350 g / dl and transferrin saturation (TS) < 15 %.
  ii. ACD – Serum iron levels < 60 – 180 g / dL, TIBC < 250 - 450 g / dl and TS < 25 – 75 %.
  iii. BTT - When HbA2 level was > 4 % high-pressure liquid chromatography (HPLC) – variant analysis using BIO –RAD D-10TM haemoglobin testing system).

Statistical Analysis

- Student t-test was applied to compare the groups.
- The level of significance was set at a P-value < 0.05.
- A receiver operator characteristic (ROC) curve was used to evaluate the discriminatory power of each reticulocyte parameter.
- Using Youden's J statistic a cut point was calculated for each parameter.
- Using this, sensitivity and specificity, positive and negative predictive values were obtained.

RESULTS

A total of 385 cases was included in this study, of which 229 were controls. Of the 156 cases of microcytic hypochromic anaemia studied, IDA comprised 91 cases, ACD – 50 cases, BTT – 15 cases, in descending order of frequency. Of the 50 ACD cases, 37 were associated with IDA. The control group comprised of 229 adult medical students (143 women and 103 men) median age 18.84 ± 0.98 years.

We also had 4 cases of other haemoglobinopathies, which were microcytic hypochromic, but not included in our study as the number of cases were too less to be analysed. Reticulocyte parameters in all the groups along with those of healthy controls are summarised in Table 1.

Parameters	IDA (N = 91)	ACD (N = 50)	BTT (N = 15)	Control (N = 229)
RETIC Mean ± SD	1.72 ± 0.76	2.51 ± 1.74	1.87 0.87	1.15 ± 0.38
Normal range	(1.57 - 1.88)	(2.02 - 2.99)	(1.43 - 2.31)	(1.10 - 1.20)
Range	(0.48, 4.17)	(0.35, 8.30)	(1.08, 4.34)	(0.40, 2.65)
IRF Mean ± SD	0.46 ± 0.08	0.47 ± 0.13	0.47 ± 0.08	0.32 ± 0.06
Normal range	(0.44 - 0.48)	(0.44 - 0.50)	(0.43 - 0.51)	(0.32 - 0.33)
Range	(0.23, 0.64)	(0.12 - 0.70)	(0.36, 0.62)	(0.16, 0.52)
MRV Mean ± SD	94.76 ± 9.33	101.89 ± 13.43	90.36 ± 9.11	101.91 ± 10.65
Normal range	(92.8 - 96.7)	(98.2 - 105.6)	(85.70 - 95.0)	(100.5 - 103.3)
Range	(75.7, 122.2)	(67.1, 132.8)	(78.2, 116.4)	(1.04, 134.1)
MRV2MCV Mean ±	1.48 ± 0.14	1.43 ± 0.19	1.47 ± 0.16	1.20 ± 0.12
SD Normal range	(1.45 - 1.51)	(1.37 - 1.48)	(1.38 - 1.55)	(1.19 - 1.22)
Range	(1.16, 1.80)	(0.91, 2.14)	(1.32, 2.00)	(0.01, 1.37)
MSCV Mean ± SD	66.4 ± 7.9	72.2 ± 7.4	67.77 ± 6.23	79.98 ± 4.81
Normal range	(64.8 - 68.1)	(70.2 - 74.3)	(64.6 - 70.9)	(79.4 - 80.6)
Range	(54.3, 95.0)	(50.7, 83.9)	(56.9, 84.4)	(66.1, 98.8)
RSF Mean ± SD	77.99 ± 7.47	83.35 ± 8.70	74.50 ± 4.82	93.38 ± 3.86
Normal range	(76.5 - 79.5)	(82.9 - 87.8)	(72.1 - 76.9)	(92.9 - 93.9)
Range	(62.5, 97.4)	(57.7, 101.6)	(66.0, 82.2)	(83.8, 114.5)
LHD Mean ± SD	43.30 ± 28.11	23.24 ± 26.87	21.94 ± 15.64	3.53 ± 2.03
Normal range	(37.5 - 49.1)	(15.8 - 30.7)	(14.0 - 29.9)	(3.3 - 3.8)
Range	(3.6, 99.2)	(0.1, 100)	(6.4, 72.2)	(0.5 - 11.9)
MAF Mean ± SD	5.43 ± 1.77	5.73 ± 1.33	6.78 ± 1.05	11.58 ± 1.41
Normal range	(5.1 - 5.8)	(5.4 - 6.1)	(6.2 - 7.3)	(11.4 - 11.8)
Range	(1.9, 9.3)	(3.4, 8.2)	(4.7, 8.4)	(2.7, 15.3)

Table 1. Reticulocyte Indices of Microcytic Hypochromic Anaemia and Control Group

As shown in Table 1, the normal range (also known as 95 % confidence interval) lies tightly around the mean. However, the data contains outliers of extreme value, thus increasing the standard deviation considerably.

Iron Status	IDA [N:91]	ACD with IDA [N:37]	ACD [N:13]	BTT [N:15] HbA2: 4.9 ± 0.98
Se Iron[N] 70 – 180 mg / dl	20.74 ± 14.57	19.27 ± 11.23	36.12 ± 10.99	91.4 ± 76.04
TIBC [N] 255 – 450 µg / dl	433.88 ± 62.77	251.95± 73.94	177.65 ± 71.21	300.45 ± 66
% TSAT [N] 25 –75 %	5.01 ± 3.75	7.94 ± 4.07	25.10 ± 9.93	29.71 ± 23.75

Table 2. Iron Status Used to Classify the Patients in Different Groups

In our study, LHD, RSF, MSCV and MRV was found to be good predictors for differentiating IDA from ACD. LHD had a sensitivity of 70.3 % and specificity of 72.0 % and was a very good predictor to differentiate IDA from ACD. It was also helpful in differentiating IDA and ACD groups from control group. MRV was found to have a sensitivity of 78 % and a specificity of 62 %. It was found to be helpful in differentiating IDA, ACD and BTT and was also helpful in differentiating each of them from the control group.

MSCV had a sensitivity of 79.1 % and a specificity of 74.0 % and was helpful in differentiating each group from control groups. RSF had a sensitivity of 82.4 % and a specificity of 64 % and it was helpful in differentiating ACD from IDA, but not helpful in differentiating them from control group.

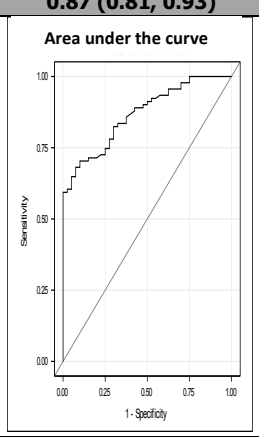
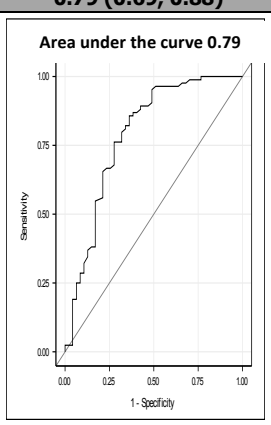
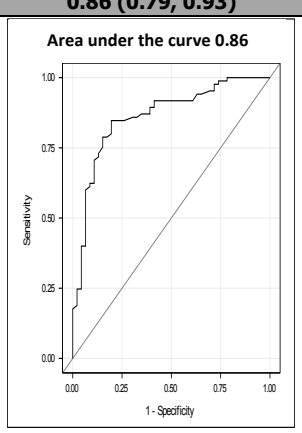
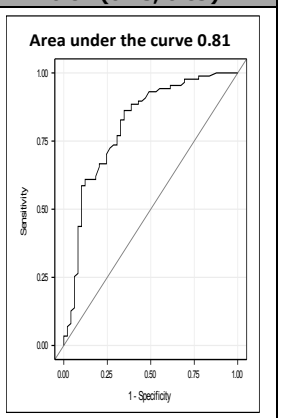
	LHD	MRV	MSCV	RSF
Area under the curve: AUC (95 % CI)	**0.87 (0.81, 0.93)**	**0.79 (0.69, 0.88)**	**0.86 (0.79, 0.93)**	**0.81 (0.73, 0.89)**
				
Cut point for ACD	≤28.3	≥98.8	≥70.0	≥83.6
Odds ratio (95 % CI)	31.86 (9.16, 110.78)	9.0 (3.98, 20.34)	15.65 (6.69, 36.65)	10.67 (4.68, 24.30)
Sensitivity (95 % CI)	0.94 (0.83, 0.99)	0.60 (0.45, 0.74)	0.74 (0.59, 0.85)	0.64 (0.49, 0.77)
Specificity (95 % CI)	0.67 (0.56, 0.77)	0.86 (0.77, 0.92)	0.85 (0.76, 0.91)	0.86 (0.77, 0.92)
Positive PV (95 % CI)	0.61 (0.49, 0.72)	0.69 (0.54, 0.83)	0.73 (0.58, 0.84)	0.71 (0.56, 0.84)
Negative PV (95 % CI)	0.95 (0.87, 099)	0.79 (0.70, 0.87)	0.86 (0.77, 0.92)	0.81 (0.72, 0.88)
Pre-test (95 % CI)	0.35 (0.28, 0.44)	0.35 (0.28, 0.44)	0.35 (0.28, 0.44)	0.35 (0.28, 0.44)
Post-test (95 % CI)	0.61 (0.49, 0.72)	0.69 (0.54, 0.83)	0.73 (0.58, 0.84)	0.71 (0.56, 0.84)
LR + (95 % CI)	2.85 (1.89, 4.30)	4.29 (91.96, 9.25)	4.93 (2.46, 9.44)	4.57 (2.13, 9.63)

Figure 1. ROC Depicting the Aptness of Each Parameter to Differentiate ACD from IDA

DISCUSSION

Microcytic hypochromic anaemia is the type of anaemia in which the circulating RBCs are smaller than the usual size of RBCs (microcytic) and have decreased red color (hypochromic). Most common cause of this type of anaemia is decreased iron reserves of the body which may be due to multiple reasons. This may be due to decreased iron in the diet, poor absorption of iron from the gut, acute and chronic blood loss, increased demand of iron in certain situations like pregnancy or recovering from a major trauma or surgery.

According to epidemiologic data from World Health Organization (WHO), 24.8 % of the human population is currently suffering from anaemia out of which a major portion is due to iron deficiency anaemia. Hypochromic

microcytic anaemia is more common in perimenopausal females because they lose blood with each menstrual cycle. Among the female population, almost 41 % of all pregnant females suffer from anaemia while among non-pregnant perimenopausal females, 30 % females are struggling with symptoms of anaemia. The male population is usually resistant to anaemia due to circulating testosterone levels. However, 12.7 % adult males are also globally affected with anaemia. After the female population, pre-school aged children suffer the most from anaemia because of lack of iron in their primary diet. Human milk contains 0.3 mg / L iron which does not provide enough iron. On the other hand, cow's milk contains double the amount of iron, but that iron has poor bioavailability.[14]

Among the newer reticulocyte parameters, low hemoglobin density (LHD) is a new parameter provided by Beckman-Coulter derived from the mean cell haemoglobin concentration. Our study investigated the reliability of LHD for the assessment of iron status in the presence of inflammation. Healthy subjects (N = 229) and patients with iron deficiency (IDA, N = 91) and anaemia of chronic disease (ACD, N = 50) were analysed using Beckman Coulter DxH80 and receiver operating characteristic (ROC) curve analysis were applied.

LHD was found to be a very good predictor to differentiate IDA from ACD and was also helpful in differentiating IDA and ACD groups from control group. In addition with the complete blood cell counts and iron parameters, LHD could enable the diagnosis to be made rapidly and accurately.

By correlating the red cell indices and biochemical markers of negative iron balance, including reduced serum ferritin, serum iron and transferrin saturation (Ioannou et. al. 2002) uncomplicated IDA could be recognised. Functional iron deficiency is defined as an imbalance between the iron needed for erythropoiesis and the iron supply, with the latter not maintained at sufficient rate. The accurate assessment of iron status in chronically ill patients was challenging.

Red blood cell size factor (RSF) is another new parameter provided by Beckman-Coulter which joins together the volume of erythrocytes and the volume of reticulocytes. The diagnostic usefulness of RSF was evaluated on a validation group which included 220 consecutive patients with anaemia by Onofrio G, Chirillo R et al. This study showed a very good level of agreement between RSF and CHr (reticulocyte haemoglobin content). Both were suitable parameters for the study of erythropoiesis. Our study showed that RSF was helpful in differentiating ACD from IDA, but not helpful in differentiating them from control group.

Studies have generated algorithms involving both these parameters and a MCV - MSCV > 10 and MRV - MSCV < 25 showing a good accuracy as a screening for cases of hereditary spherocytosis. It also helps to differentiate these conditions from other common causes of spherocytosis, i.e. autoimmune haemolytic anaemia (AIHA) and ABO incompatibility.[15]

Few studies are available on the clinical usefulness of mean reticulocyte volume. In subjects with depleted iron stores, this index increases rapidly following iron therapy and decreases equally as rapidly with the development of iron-deficient erythropoiesis. The reticulocyte volume decreases dramatically and reticulocytes are smaller than the circulating RBCs, in nutritional macrocytosis after therapy with vitamin B12 and / or folic acid.[16]

The main limitation of the use of these indices is the difficulty to compare numeric results obtained from the analysers of different manufacturers.

CONCLUSIONS

Thus in our study, we came to a conclusion that the new reticulocyte parameters were useful for evaluation of iron status and diagnosing iron deficiency anaemia. They also are reliable parameters for recognising subsets of anaemic patients thereby improving the management of anaemia.[17] It was seen that RSF was also a useful parameter for study of erythropoiesis. The advantage is that these tests can be performed simultaneously with routine blood counts without an additional increase in cost.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
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