

EVALUATION OF ANAEMIA USING RED CELL AND RETICULOCYTE PARAMETERS USING AUTOMATED HAEMATOLOGY ANALYSER

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ABSTRACT

Use of current models of Automated Haematology Analysers help in calculating the haemoglobin contents of the mature Red cells, Reticulocytes and percentages of Microcytic and hypochromic Red cells. This has helped the clinician in reaching early diagnosis and management of Different haemopoietic disorders like Iron Deficiency Anaemia, Thalassemia and anaemia of chronic diseases.

AIM

This study is conducted using an Automated Haematology Analyser to evaluate anaemia using the Red Cell and Reticulocyte parameters. Three types of anaemia were evaluated; iron deficiency anaemia, anaemia of long duration and anaemia associated with chronic disease and Iron deficiency.

MATERIALS AND METHODS

The blood samples were collected from 287 adult patients with anaemia differentiated depending upon their iron status, haemoglobinopathies and inflammatory activity. Iron deficiency anaemia (n=132), anaemia of long duration (ACD), (n=97) and anaemia associated with chronic disease with iron deficiency (ACD Combi), (n=58). Microcytic Red cells, hypochromic red cells percentage and levels of haemoglobin in reticulocytes and matured RBCs were calculated. The accuracy of the parameters was analysed using receiver operating characteristic analyser to differentiate between the types of anaemia.

OBSERVATIONS AND RESULTS

There was no difference in parameters between the iron deficiency group or anaemia associated with chronic disease and iron deficiency. The hypochromic red cells percentage was the best parameter in differentiating anaemia of chronic disease with or without absolute iron deficiency with a sensitivity of 72.7% and a specificity of 70.4%.

CONCLUSIONS

The parameters of red cells and reticulocytes were of reasonably good indicators in differentiating the absolute iron deficiency anaemia with chronic disease.

KEYWORDS

Automated, Anaemia, Iron Deficiency, Red cells, Reticulocytes, Haemoglobinopathies.

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INTRODUCTION: The current models of Automated Haematology Analysers help in calculating the haemoglobin contents of the mature Red cells, Reticulocytes and percentages of Microcytic and hypochromic Red cells. These new Analysers have been used in the diagnosis of iron deficiency Anaemia, beta thalassemia carriers and anaemia of chronic disease.^{1,2,3} As the clinical approach to these conditions is entirely different the differentiation between them remains most important.^{4,5}

Due to short life span of Reticulocytes (1 to 2 days), the haemoglobin content of young red cells remains a good measurement of iron deficiency erythropoiesis.⁶ Reticulocyte haemoglobin content represents real-time information on the synthesis of young RBCs in the marrow.

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The other parameters are the percentage of red cells with haemoglobin content 17 pg or less, and the percentage of red cells with a volume of less than 60 fL (% Micro R),¹ which corresponds to a sub-population of mature red cells exhibiting evidence of insufficient iron content. Among the various causes of anaemia with which the patients get admitted is anaemia associated with chronic inflammation, infection or malignancy.

Presence of inflammatory cytokines causes failure in incorporation of iron in the red cell progenitors even though there is abundant stainable iron in the bone marrow. This is also called as hyporegenerative anaemia.⁷ Reduced concentrations of circulating iron and normal or increased iron stores characterise a state of functional iron deficiency.⁸

Anaemia of inflammation can be associated with absolute iron deficiency such as generally in patients with inflammatory disease and chronic blood loss. Differentiation between these two conditions is very important in the treatment but their differentiation is difficult in clinical practice by using conventional bio markers such as ferritin concentration and transferrin saturation.⁹

In such situations, transferrin receptor/log ferritin ratio calculated by automated haematology analysers may be useful in distinguishing these two conditions.¹⁰ In the present study, the utility of automated haematology analysers which give effectively new laboratory parameters related to mature RBCs and Reticulocytes, helping in differentiation of anaemia of iron deficiency and anaemia of longstanding duration and anaemia associated with chronic diseases was evaluated.

MATERIALS AND METHODS: The present study was conducted in the department of clinical pathology of Kannur Medical College, Anjarakandy, Kannur, Kerala. It was approved by the Ethical Committee of the Institute. 287 blood samples were selected from the samples arriving at the central laboratory of the Institute from various departments of the Medical College Hospital and so informed consent was waived off. The patient's samples showing Haemoglobin less than 12.0 g/dL for females and less than 14.0 g for males were selected from the workload of blood samples arriving at central laboratory with a requisition of investigation to exclude anaemia.

Inclusion Criteria: Patient's samples selected when Haemoglobin is <12 g/dL in females and < 14.0 g/dL in males.

Exclusion Criteria: Patients who have undergone blood transfusions within three months are excluded from the study. Patients with β -thal associated with other kinds of anaemia, patients with reticulocytosis or pancytopenia are excluded. Patients who are on Iron therapy are excluded from the study. These samples were classified depending upon their iron status analysis (Using commercial kits from Roche Diagnostics, Germany): Iron deficiency was labelled when the serum iron (SI) levels were <45 mg/dL for males and <30 mg/dL for females and transferrin saturation <15% for males and females, serum ferritin <30 μ g/L for males and <13 μ g/L for females.

The samples were labelled as anaemia due to iron deficiency in chronic disease when SI levels were normal or less than normal (40-160 mg/dL and 30-160 mg/dL in males and females respectively), transferrin saturation was normal or less than normal (3-50%), serum ferritin levels were normal or high (30-400 μ g/L and 13-150 μ g/L for males and females respectively and C-reactive protein >5 mg/L (Tin-Quant C-reactive Protein, Roche Diagnostics, Germany).

Soluble transferrin receptor (sTfR) levels (Roche Diagnostics, Germany) were measured in all samples, and the sTfR/log ferritin ratio was used to identify iron deficiency in patients with Iron Deficiency Anaemia. Patients with Iron Deficiency anaemia showing sTfR/log ferritin >2.06 or sTfR >3.71 μ g/mL (Cut-off values indicative of iron deficiency in our laboratory) were classified as Iron deficiency following chronic diseases. Blood samples showing normal Haemoglobin levels were used as a control group consisting of apparently healthy persons, including absence of acute inflammation/infection, with normal haematologic findings, and C-reactive protein <5 mg/L.

The healthy individuals were students and laboratory staff, all of whom had donated blood samples on a voluntary basis. Determination of red cell and reticulocyte parameters was performed using a Beckman-Coulter LH 750 automated haematological analyser which provided the following parameters: Ret-He, % MicroR, haemoglobin content of red cells obtained from optical counting (RBC-He), and % Hypo He. The MHe index was calculated as % MicroR - % HypoHe. The Mann-Whitney test was used to compare the groups. A receiver operator characteristic (ROC) curve was used to evaluate the accuracy of the parameters to differentiate between the different types of anaemia. The level of significance was set at a p-value <0.05. Data were analysed using SPSS for Windows, Version 13.0 (SPSS Inc., Chicago, IL, USA).

OBSERVATIONS AND RESULTS: In the present study, the samples of patients with Iron deficiency anaemia (IDA) (n=132), anaemia of long duration (n=97) and anaemia associated with chronic disease with iron deficiency (n= 58).

Parameter	IDA a	ACD b	ACD Combi	Control Group
N	132	97	58	76
Age				
Median/Range	41/18.5-71	36/19-67	39/22-70	41.5/18-77
Sex				
Male/Female	87/45	38/59	32/26	31/45
Haemoglobin (G/dL)				
Median/Range	7.4/6.5-11.2	9.8/8.2-11.6	9/6.8-10.4	13.5/12.4-14.4
MCV				
Median/Range	74.2/60.2-77.5	73.5/56.5-81.4	79.2/60.2-96.4	88.6/82.4-94.5
MCH/(fl)				
Median/Range	24.5/19.8-25.2	26.2/23-27.4	27.8/26.2-28.2	30/28.2-30.2
MCH (pg)				
Median/Range	21.5/16.0-25.6	25.2/17.2-28.8	28.8/16.5-26.8	30.2/26.2-33.2
RDW				
Median/Range	15.4/12.4-20.2	16.2/13.2-19.6	15.5/13.0-27.8	13.2/12.6-15.5

Table 1: Showing the Demographic Data Laboratory Findings of Patients and Control Group (n=287)

IDA: Iron Deficiency anaemia. **ACD:** Anaemia of Chronic Disease. **ACD Combi:** Anaemia of Chronic Disease associated with iron deficiency. **CG:** Control Group. **RDW:** Red cell distribution width. a) p=0.032 and b) p=0.024

Parameters	IDA a	ACD b	ACD Combi	CG
N	132	97	58	76
Ret-He (pg)				
Median/range	24.0/15.5-30.5	26.0/18.8-36.0	28.7/22.0-39.0	35.1/32.0-39.5
RBC-He (pg)				
Median/Range	223.5/17.2-36.3	27.0/19.5-30.0	28.8/20.4-24.9	31.9/28.2-35.6
Hypo He (%)				
Median/Range	6.2/0.5-44.5	4.2/1.4-30.5	1.3/0.4-16.2	0.2/0.1-0.9
Micro R (%)				
Median/Range	12.5/0.5-47.5	14.5/0.6-56.5	5.5/1.4-47.5	1.6/0.8-4.0
MHe				
Median/Range	5.8/9.8-22.6	7.2/0.0-28.5	4.05/0.5-35.5	1.15/0.3-3.7

Table 2: Showing the Iron Status Measurements Used to Classify the Patients in the Different Groups (N=287)

SI: Serum iron, **TS:** Transferrin saturation; **SF:** Serum ferritin; **sTfR:** Soluble transferrin receptor.

Parameters	IDA a	ACD b	ACD Combi	CG
N	132	97	58	76
Ret-He (pg)				
Median/range	24.0/15.5-30.5	26.0/18.8-36.0	28.7/22.0-39.0	35.1/32.0-39.5
RBC-He (pg)				
Median/Range	223.5/17.2-36.3	27.0/19.5-30.0	28.8/20.4-24.9	31.9/28.2-35.6
Hypo He (%)				
Median/Range	6.2/0.5-44.5	4.2/1.4-30.5	1.3/0.4-16.2	0.2/0.1-0.9
Micro R (%)				
Median/Range	12.5/0.5-47.5	14.5/0.6-56.5	5.5/1.4-47.5	1.6/0.8-4.0
MHe				
Median/Range	5.8/9.8-22.6	7.2/0.0-28.5	4.05/0.5-35.5	1.15/0.3-3.7

Table 3: Showing Reticulocyte and Red Cell Indices for Patient and Control Groups (n-287)

Ret-He: Reticulocyte haemoglobin content; **RBC-He:** Red blood cell haemoglobin content; **HypoHe:** Percentage of hypochromic red cells; **MicroR:** Percentage of Microcytic red cells; **MHe:** MicroR – HypoHe index.

Mann–Whitney test was applied for comparison between groups.

A: $p \leq 0.01$ (ACD × ACD Combi).

B: $p < 0.001$ (ACD × CG).

Applying the Mann–Whitney test in the present study, there was no significant difference between the IDA and ACD Combi groups in respect to all parameters. Comparing the ACD and ACD Combi groups, the RBC-He and Ret-He were significantly lower for the ACD Combi group (p -value =0.016 and p -value=0.003, respectively). Similarly, the %MicroR, %HypoHe and the value of MHe were significantly higher in the ACD Combi group (p -value=0.001, p -value=0.003 and p -value=0.014, respectively).

Although the ACD group showed sTfR/log ferritin values below the cut-off limit indicative of iron deficiency, the Ret-He and RBC-He values were significantly reduced when compared to the control group. However, the %HypoHe, %MicroR and MHe were higher (p -value <0.001 for all). Values below the cut-off of 13.8 showed a sensitivity of 96.2% and specificity of 92.7% in identifying IDA patients.

A good performance was seen for the %MicroR (AUC: 0.886; CI 95%: 0.810–0.963), and values <25.0% gave sensitivity of 84.6% and specificity of 78.0% in detecting iron deficiency.

When the ROC curve was applied to the ACD and ACD Combi groups, the best performance was seen with the %HypoHe parameter, although with a moderate AUC value

(AUC: 0.785; 95% CI: 0.661–0.909; sensitivity 72.7%, and specificity 71.4%; cut-off: 1.8%).

The best parameter to distinguish IDA from ACD was the %HypoHe (AUC: 0.835; 95% CI: 0.737–0.933). A value for %HypoHe <2.45% had a sensitivity of 75.4% and specificity of 70.4% in identifying ACD. The AUCs were lower for RBC-He (AUC: 0.809; 95% CI: 0.696–0.922), Ret-He (AUC: 0.780; 95% CI: 0.661–0.899), and %MicroR (AUC: 0.785; 95% CI: 0.662–0.908). The capacity of the tests in discriminating IDA from ACD Combi was not satisfactory as the AUC was lower than 0.700 for all parameters.

DISCUSSION: Many authors have tested the diagnostic performance of reticulocyte parameters, especially for the diagnosis of iron deficiency in patients submitted to dialysis.^{10,11,6} Measuring the reticulocyte content is helpful in detecting the earliest stages of iron deficiency, before anaemia develops.^{6,12,13} In conditions other than iron deficiency, the reduction in reticulocyte haemoglobin was observed in haemoglobinopathies.^{14,15} In other studies also using reticulocyte parameters were used in diagnosing IDA and ACD.^{2,8,16} Recent studies showed that classic indices like mean cell haemoglobin and mean cell haemoglobin concentration are better to differentiate between IDA and ACD especially in geriatric patients rather than Ret-He.⁵

In the present study, the Ret-He values were lower for IDA and ACD and the accuracy to identify both the anaemias was moderate and less than using %HypoHe. Ret-He parameter was highly useful in cases of chronic rheumatic disease and anaemia. Similarly, the predictive value of Ret-He tested in response to oral iron therapy responsiveness was supportive.¹⁷ Besides Ret-He, Additional red blood cell

parameters like %MicroR, and %HypoHe, are tested either alone or in combination.¹⁸

The MHe index was first proposed and tested by Urrechaga et al.¹⁹ The performance of this index was better (Sensitivity 98.0% and specificity 95.9) than other published indices. Very few reports exist about the efficiency of other red cell parameters in patients with anaemia of inflammation associated with absolute iron deficiency. The clinical utility of the determination of the %Hypo has long been recognised in differentiating between iron-deficient and iron-sufficient patients with chronic kidney disease who receive erythropoietin stimulating agents.²⁰ In patients with ACD, the sTfR/log ferritin ratio values were calculated to identify iron deficiency. Unlike SI, transferrin and ferritin measurements the measurement of sTfR has been considered a good indicator of functional iron status as it does not suffer the influences of systemic inflammation.^{21,22}

Thus, when there is a reduction of functional iron, transferrin receptor synthesis is stimulated. Iron deficiency is characterised by an increase in sTfR levels and low ferritin values, while in anaemia of inflammation, transferrin receptor levels are only slightly affected and serum ferritin is greatly increased.²³ In clinical practice, this differentiation is important because iron supplementation is beneficial for ACD Combi, but may be deleterious for ACD patients. The best parameter to distinguish ACD Combi from ACD was the %HypoHe even though the sensitivity and specificity were moderate. This is followed by %MicroR. In the present study, it was noted that there were no differences in test results between the IDA and ACD Combi groups. In contrast, there were differences observed when ACD was compared with IDA. The fact is that the absolute iron deficiency associated to ACD increases the number of microcytic and hypochromic red cells, developing cell features similar to IDA. ACD patients showed evidence of reduced iron availability for erythropoiesis, but the disturbance of the iron metabolism in functional iron deficiency was less remarkable than in the association of ACD with IDA.

CONCLUSION: In practical terms, the incorporation of new cellular indices can hasten the process of diagnosis of IDA and ACD. Consequently, target more quickly and more precisely the subsequent confirmatory examinations in order to introduce the appropriate treatment. But the difficulty in identifying absolute iron deficiency in patients with inflammatory conditions remains unaltered. Therefore, the challenges persist and other studies are needed to find a parameter with values in clinical decision making.

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