Original Article

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Pathology Section

Usefulness of Reticulocyte Haemoglobin Equivalent in the Evaluation of Iron Deficiency Status: A Cross-sectional Study

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ABSTRACT

Introduction: Iron deficiency is the most frequent cause of nutritional anaemia. Evaluation of parameters like serum ferritin and iron is critically important for evaluation of the iron status of patients and diagnosis of Iron Deficiency Anaemia (IDA). But in inflammatory disorders, ferritin concentration may be normal or increased due to the acute phase response, resulting in inaccurate interpretation of the iron status. Similarly, the serum iron levels may be high in cases of thalassaemia with combined iron deficiency. Reticulocyte Haemoglobin equivalent (RET-He) which correlates to the iron content in reticulocytes is effective, convenient and provides useful information for the immediate diagnosis and treatment of iron deficient states.

Aim: To evaluate the diagnostic performance of RET-He level by comparing it with the iron status of the patients.

Materials and Methods: This cross-sectional study was done in Department of Pathology at Tagore Medical College and Hospital, Chennai, Tamil Nadu, India, from December 2020 to November 2021. The complete blood count, RET-He, serum iron and serum ferritin levels of 406 patients with microcytic hypochromic anaemia and dimorphic anaemia were reviewed and RET-He levels were compared with the serum iron and ferritin levels. The p-value was calculated using Statistical Package for Social Sciences (SPSS) IBM software version 28.0. The sensitivity of RET-He and serum ferritin levels in evaluating iron deficiency was calculated.

Results: Low RET-He was observed in 394 cases, low serum iron in 401 cases and reduced serum ferritin in 376 cases. Total 34 cases of IDA with concomitant inflammation showed low RET-He and serum iron, despite normal to high serum ferritin levels. Five cases of iron deficiency with concomitant haemoglobin defect also showed low RET-He levels and serum ferritin, whereas the serum iron was raised. Low RET-He was observed in 17 cases of dual deficiency anaemia i.e., iron deficiency along with vitamin B12/folate deficiency.

Conclusion: It was concluded that measurement of the reticulocyte haemoglobin content provides useful information for the diagnosis, treatment and monitoring of iron deficient states.

Keywords: Dual deficiency anaemia, Erythropoiesis, Microcytic hypochromic anaemia, Serum ferritin

INTRODUCTION

Iron deficiency is the most frequent cause of nutritional anaemia. Evaluation of parameters like serum ferritin and iron is critically important for evaluation of the iron status of patients and diagnosis of Iron Deficiency Anaemia (IDA). But in inflammatory disorders, ferritin concentration may be normal or increased due to the acute phase response, resulting in inaccurate interpretation of the iron status. The recent automated haematology analysers measure Reticulocyte haemoglobin equivalent (RET-He) in the same sample used for complete blood count tests, which correlates to the iron content in reticulocytes. It is a reliable marker of cellular haemoglobin content and provides information about the current availability of iron in erythropoiesis [1,2]. Studies show that the reticulocyte haemoglobin content is effective, convenient method and helps in the immediate diagnosis and treatment of iron deficient states [3,4].

In this study, the diagnostic performance of RET-He provided by Sysmex XN1000 analyser was evaluated by comparing it with the iron status of the patients. The RET-He is not used widely as a routine test and its significance is largely under-rated. This study emphasises on the use of RET-He in determination of iron deficiency in various conditions like inflammation, dual deficiency and concomitant haemoglobin defect. The RET-He is not affected by inflammation, its determination is fully automated and can be performed on the same sample as for Complete Blood Count (CBC), hence may alleviate the need for invasive procedures like bone marrow aspiration. Inclusion of RET-He in routine CBC can help in identifying early iron deficiency states and quicker monitoring of iron therapy. The aim of this study was to evaluate the diagnostic performance of RET-He level by comparing it with the iron status of the patients.

MATERIALS AND METHODS

This cross-sectional study was done in Department of Pathology at Tagore Medical College and Hospital, Chennai, Tamil Nadu, India, from December 2020 to November 2021. The CBC, RET-He, serum iron and serum ferritin levels of 406 patients with microcytic hypochromic anaemia and dimorphic anaemia were reviewed.

Inclusion criteria: Patients above 12 years of age and all samples, with peripheral blood picture of microcytic hypochromic anaemia and dimorphic anaemia were included in the study.

Exclusion criteria: Post-transfusion samples and paediatric samples (ages <12 years) were excluded.

Procedure

The complete blood count of these samples were reviewed and RET-He was done in Sysmex XN 1000 analyser. The RET-He levels were compared with the corresponding serum iron and ferritin levels. C-Reactive Protein (CRP) levels were done in cases of IDA with concomitant inflammation. Soluble Transferrin Receptor (sTFR) Assay was done in cases of iron deficiency with concomitant haemoglobin defect as the serum iron is raised in cases of haemoglobin defect.

STATISTICAL ANALYSIS

The p-value of significance was calculated by using Statistical Package for Social Sciences (SPSS) IBM software version 28.0. A paired samples t-test was done. The sensitivity of RET-He and serum ferritin levels in evaluating iron deficiency was calculated.

RESULTS

In this study, 389 cases of microcytic hypochromic anaemia (Haemoglobin <11 mg/dL, mean corpuscular volume <80 fL) and 17 cases of dimorphic anaemia were evaluated for low RET-He (<29 pg) and low Iron status of the patient (serum iron <65 µg/dL, serum ferritin <100 ng/mL). The mean age group of the patients were 48.6 years (ranging from 16 to 88 years) with 266 females and 123 males.

Low RET-He was observed in 394 cases, low serum iron in 401 cases and reduced serum ferritin in 376 cases. Total 34 cases of IDA with concomitant inflammation (confirmed by raised CRP levels) showed low RET-He and serum iron, despite normal to high serum ferritin levels. Five cases of iron deficiency with concomitant haemoglobin defect also showed low RET-He levels and serum ferritin, whereas the serum iron was raised. The low iron status was confirmed by Soluble Transferrin Receptor (sTFR) Assay in these five cases. Low RET-He was also observed in 17 cases of dual deficiency anaemia i.e., iron deficiency along with vitamin B12/folate deficiency. The comparison between RET-He level, serum iron and serum ferritin levels is shown in [Table/Fig-1]. The sensitivity of RET-He and serum ferritin is tabulated in [Table/Fig-2]. The p-value of RET-He was significant, in all the scenarios as depicted in [Table/Fig-3].

Number of cases with	Iron deficiency anaemia	Iron deficiency with concomitant inflammation	Iron deficiency with concomitant haemoglobin defect	Dual deficiency anaemia	Early iron deficiency	Total no. of cases
Reduced serum iron	309	34	0	17	41	401
Reduced reticulocyte haemoglobin	301	34	5	17	37	394
Low srerum ferritin	305	13	5	17	36	376

[Table/Fig-1]: Comparison of Ret-He with serum Iron and serum Ferritin levels.
sTFR assay confirmed the iron deficient status.

Variables	Iron deficiency anaemia	Iron deficiency with concomitant inflammation	Iron deficiency with concomitant haemoglobin defect	Dual deficiency anaemia	Early iron deficiency
Reticulocyte haemoglobin equivalent	97.4%	100%	100%	100%	90.2%
Srerum ferritin	98.7%	38.2%	100%	100%	87.8%

[Table/Fig-2]: Sensitivity of RET-He and Srerum ferritin in evaluating Iron deficiency.

Variables	No. of cases with Iron deficiency	No. of cases with reduced RET-He	p-value**
Iron deficiency anaemia	309	301	**<0.001**
Iron deficiency with concomitant inflammation	34	34	**<0.001**
Iron deficiency with concomitant haemoglobin defect	5	5	**0.005**
Dual deficiency anaemia	17	17	**0.023**
Early iron deficiency	41	37	**0.021**

[Table/Fig-3]: Significance of RET-He in the evaluation of Iron deficiency.
**Paired samples t-test was done; p-value <0.05 was considered as statistically significant

DISCUSSION

Iron Deficiency (ID) is one the most common cause of nutritional anaemia. Diagnosis of IDA depends on the applied biomarkers of ID, and Transferrin Saturation Coefficient (TSAT) or ferritin when used alone may lead to diagnostic difficulties, especially in cases of inflammation [5]. The RET-He provides information about the current availability of iron in erythropoiesis. The RET-He is independent of the acute phase and can be determined within a few minutes by a blood count [4,8].

Due to the approximately 120 day lifetime of erythrocytes, iron deficiency and changes in the iron status of erythropoiesis can first be recognised at a relatively late stage using classical haematological parameters, such as haemoglobin, mean corpuscular volume, mean cellular haemoglobin content and also with determination of hypochromic erythrocytes. Reticulocytes which are the precursors of mature erythrocytes, enter the peripheral blood from bone marrow. They usually mature within 2 days to mature erythrocytes. Hence, the estimation of reticulocyte number provides a timely information about erythropoiesis. As the haemoglobin content of reticulocytes reflects the actual iron metabolism of erythropoiesis, it provides an assessment of the quality of the cells. Thereby any changes in the iron status of erythropoiesis can be detected earlier rather than the haemoglobin content of mature erythrocytes, i.e. the mean cellular haemoglobin content [1,6].

The evaluation of RET-He can be carried out as a routine preoperative marker of latent anaemia to identify at risk patients. In perioperative cases, patients with iron deficiency can be treated proactively at an early stage, hence preventing complications and extended hospital stays [1]. The RET-He can be also be used as a routine screening test to detect early iron deficiency in blood donors. This could provide an opportunity to make appropriate and timely interventions like dietary changes or drug supplementation [7]. Studies indicate that for ID and IDA in children, RET-He can efficiently be used as a single screening parameter without the consideration of other iron parameters [8].

The RET-He is a real time indicator of iron supply (haemoglobinization) to the developing RBC's and the earliest marker of response to iron therapy [9]. It is useful in screening of iron deficiency, diagnosis of iron deficiency anaemia, and diagnosis of functional iron deficiency anaemia in acute or chronic diseases or inflammation and helps to monitor iron therapy [10]. It may improve the targeting of iron supplementation programs in resource-limited countries [11-14].

Limitation(s)

Limited availability of data for RET-He levels after commencement of iron therapy. Out of the 406 cases studied, the RET-He levels after iron therapy was available only in 24 cases. The RET-He levels showed corresponding increase with the raise in haemoglobin and serum iron levels. However, the duration of iron therapy varied in each case and regular monitoring was not possible due to various factors like patient non compliance, missed review visits. Hence, the data has not been included in this study. Specificity could not be calculated as the negative cases (non iron deficient) were not included in the study.

CONCLUSION(S)

Diagnosis of IDA depends on biochemical parameters such as TSAT, hepcidin or ferritin which when used alone may lead to diagnostic difficulties. Using RET-He to evaluate iron deficient state in patients with anaemia contributes in improving the diagnosis of IDA and early iron deficiency, especially in the presence of confounding factors like inflammation, combined iron deficiency and thalassaemia, where RET-He is highly preferred. In the present study, RET-He associates

significantly with the iron status of patients. Hence, it is concluded that measurement of the reticulocyte haemoglobin content provides useful information for the diagnosis, treatment and monitoring of iron deficient states. It is easy to analyse, less time consuming and less expensive.

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