بسم الله الرحمن الرحيم
Omdurman Islamic University Journal of Applied Sciences
(OIUJ)-(ASC)
مجلة جامعة أم درمان الاسلامية للعلوم التطبيقية
https://journal.oiu.edu.sd/index.php/oiuj
https://doi.org/10.52981/oiuj
2025; 21(1):16-26
ISSN: 5361-1858

Clinical Significance of Reticulocyte Maturity Indices among Iron Deficiency anemia patients in Khartoum-Sudan

Huda Abdalla Mohammed Nour Mohammed Ali [1] , Nour Mahmoud Abdelateif Ali[2]

[1] Department of Hematology, Faculty of Medical Laboratory Sciences, Alneelain University, Khartoum, Sudan.

[2] Department of Hematology, Faculty of Medical Laboratory Sciences, Alneelain University, Khartoum, Sudan.

To cite this article:

Huda Abd Allah Mohammed Nour Mohammed Ali 1 , Nour Mahmoud Abdelateif Ali2, Clinical Significance of Reticulocyte Maturity Indices among Iron Deficiency anemia patients in Khartoum-Sudan ,Omdurman Islamic University Journal, Sudan

ISSN: 5361-1858

https://doi.org/10.52981/oiuj.v1i2.1726

Abstract

Background

Anemia is described as hemoglobin (Hb) level below normal but the criteria vary according to age and gender. Red cell parameters and reticulocyte indices play an essential role in differential diagnosis of anemia and moreover in its treatment.

.**Aim**

This study aim to evaluate the diagnostic significance of reticulocyte maturity indices in iron deficiency anemia.

Patients and methods

This study was conducted in the period from September 2021 to September 2022 at Fedail Hospital- Khartoum state, Sudan. Venous 3ml of blood was collected from patients under the study and dispensed in (EDTA) container for determination. Automated RC Mindray BC- 6800 was used to analyze the blood samples.

Results

The results revealed that the patients with IDA had significantly lower mean hemoglobin (7.89 g/dl) Significant differences were noted in terms of low, medium & high fluorescence ratios in both groups ($p<0.05$).

Conclusion

Our findings suggest that medium fluorescence ratio and high fluorescence ratio were higher among Sudanese patients with iron deficiency anemia. Thus, the reticulocyte indices may help in monitoring of effective erythropoiesis and serve as early markers of iron deficiency and anemia.

Keywords: Reticulocyte maturity indices, Iron deficiency anemia

Introduction

Anemia is described as hemoglobin (Hb) level below normal but the criteria vary according to age and gender. Among children under the age of 5 years, "A secondary analysis of National Nutrition Survey Data 2011-2012" published in 2016, revealed prevalence of iron deficiency anemia as 33.2% (Habib, et al 2016). As per "World Health Organization (WHO)" in children of 6 months to 4 years, anemia is labeled as hemoglobin less than or equal to 11 g/dL (Leal, et al 2011). Peripheral blood reticulocyte count is considered helpful in diagnosis,

classification and evaluation of individuals with anaemia while it also holds the center stage for evaluation and bone marrow response to anaemia treatment (Buttarello, 2016). Iron deficiency anaemia (IDA) is among the top 5 leading causes of years spent with disability (Mawani, et al 2016). Not only IDA is a major concern in pediatric age groups, pre-menopausal and pregnant ladies, but it is also considered a clinical issue affecting individuals who present with different medical and surgical morbidities (Cappellini, et al 2020).

It needs prompt and vigilant evaluation for the diagnosis and management of IDA to improve wellbeing of the affected patients. For identifying high-risk population, early detection of anaemia and evaluation of response to therapy, new laboratory parameters are being studied (Means, 2020).

These new parameters are only available in modern automated hematology analyzers that are costly and not available at many places in developing countries like Pakistan. These new parameters are also not part of routine lab

reports at the moment due to lack of standardization and studies on large number of population samples. Reticulocytes are known to be non-nucleated immature red blood cells (RBCs) in the peripheral blood and contain reticulo-filamentous material composed of residual ribonucleic acid (RNA) (Chandran, et al 2020). For reticulocyte counts, manual method is mostly adopted in diagnostic hematology laboratories. RNA has the propensity of reacting with certain supravital stains like New Methylene Blue or Brilliant Cresyl Blue.

This results in formation of blue to purple granular or filamentous precipitate that can be viewed under microscope. Such reticulocytes are counted under high power field and reported as percentage of red blood cells (Moradabadi, et al 2019). This manual method is inherently inferior to newer automated reticulocyte analysis as it is time consuming and requires manual labor, skillful technician and microscopist. This method also does not provide any information about newer reticulocyte parameters. Recently,

Automated Reticulocyte Analysis (ARA) using flow cytometry is commonly used as a substitute to manual counts. ARA is considered a more rapid and accurate method which is easy to perform and provides number of reticulocytes count as well as numerous other indices that can help in diagnosing various pathologies and also in monitoring of bone marrow recovery (Komal, et al 2019). Many modern automated hematology analyzers are equipped with ARA.

As reticulocyte indices need standardization and labeling of reference values, their regular and proper use in clinical settings is yet to be seen. With ARA,

reticulocytes can be classified into three subpopulations as per fluorescence intensity, reflecting maturity i.e. more the RNA content and fluorescence intensity, less mature the reticulocytes. These subpopulations or indices are Low Fluorescence Ratio (LFR), Medium Fluorescence Ratio (MFR) and High Fluorescence Ratio (HFR). With this classification, intracellular RNA levels are directly linked to fluorescence intensity which show degree of maturation of reticulocytes (Urrechaga, et al 2011). International studies have demonstrated promising research in this regard and have suggested the routine use of these parameters for evaluation of anemia's including IDA. This study was aimed to evaluate diagnostic significance of measuring LFR, MFR and HFR in Sudanese iron deficiency anemic patients.

Materials and methods

This case control Laboratory based study was performed in Fadail Hospital- Khartoum state, Sudan during the period from September 2021 to September 2022. The study recruited 252 participants 126 were patients with IDA with Hb<11 g/dL and serum ferritin level <7 ng/mL. On the other hand, 126 were control group with Hb>11 g/dL and serum ferritin level >7 ng/mL. Patient's data were collected via lab records from Fedail Hospital with patients after obtaning their consent. Patients taking any iron or vitamin supplements were excluded. Approval from Al- Neelan University ethical review board was taken. 3ml of Venous blood were collected from individual under study and dispensed in Ethylenediaminetetraacetic acid (EDTA) container for determination. Automated RC Mindray BC- 6800 was used for samples analysis.

All data were analyzed using the statistical package for social sciences (SPSS, version 23) (IBM Inc, Chicago, IL, USA) All data were analyzed using the statistical package for social sciences (SPSS, version 23) (IBM Inc, Chicago, IL, USA). Descriptive statistics was done to summarize the dependent and independent variables. Chi-square test was employed to analyze the association between population characteristics and variables. Furthermore, the Spearman rank correlation coefficient was utilized to evaluate the relationship between variables with P-value<0.05 considered as significant.

Results

Out of 252 participants of the study, 126 case group and 126 control group). 37 patients were Males (29%) and 89 patients were Female (71%). The mean age was 26.40 ± 19.38. Comparison of hemoglobin level between the two groups was shown in. Mean hemoglobin level among case was (7.89 g/dl) , while among control group was (12.79g/dl).

Comparing reticulocyte parameters between case and control groups. In terms of reticulocyte indices, significant difference was observed in LFR, MFR and HRF (p<0.05) between the two groups. (Table.1)

Table.1: Comparison of reticulocyte parameters between case and control groups

Parameters	Case group	Control group	P value
Low Fluorescence Ratio (LFR, % total reticulocytes)	85.961±8.235	93.377±2.536	0.000**
Medium Fluorescence Ratio (MFR, % total reticulocytes)	11.557±5.431	6.099±2.099	0.000**
High Fluorescence Ratio (HFR, % total reticulocytes)	2.510±3.231	0.997±0.511	0.000**

Discussion

Red blood cells have an average lifespan of approximately four months, with around 1% of the erythrocyte population being renewed daily. Due to this gradual turnover, abnormalities in the hematimetric indices of anemic individuals may take weeks to become apparent. Consequently, reticulocyte indices can serve as early indicators of iron deficiency and anemia (Wollmann et al., 2014).

This study aimed to assess reticulocyte maturity indices in Sudanese patients with iron deficiency anemia (IDA). The analysis was conducted using the advanced, fully automated Mindray BC-6800 Hematology Analyzer. The results indicated a statistically significant variation in reticulocyte maturity indices. These findings suggest that the effectiveness of erythropoiesis in response to anemia can be evaluated through quantitative measurements of

reticulocyte subpopulations. Our study highlights that measuring reticulocyte maturity indices—specifically, the fractions of low-fluorescence reticulocytes (LFR), medium-fluorescence reticulocytes (MFR), and high-fluorescence reticulocytes (HFR)—is feasible in settings equipped with modern automated hematology analyzers.

One potential explanation for our results is that an increase in mature reticulocytes in the bloodstream of an IDA patient signifies the body's response to iron deficiency anemia, provided that the essential medullary and cortical components required for erythropoiesis remain intact. Anemic hypoxia triggers erythropoietin release in the bone marrow, stimulating cell proliferation and differentiation. If the concentration of reticulocytes rises in the bone marrow, their maturation will subsequently be completed in circulation. This study aimed to explore the possible correlation between reticulocyte indices (LFR, MFR, HFR) and iron deficiency anemia (Wollmann et al., 2014).

Wollmann et al. (2014) demonstrated that reticulocyte maturity indices could serve as early markers of IDA and anemia, as patients with IDA exhibited an increased mean fluorescence ratio ($p = 0.003$) and high fluorescence ratio ($p = 0.03$), findings that align with the current study. Similarly, Zhao et al. (2015) supported these results, reporting a significant decrease in the low-fluorescence reticulocyte subpopulation, while the medium- and high-fluorescence reticulocyte fractions increased in all cases of anemia compared to the control group.

Furthermore, Velasco-Rodríguez et al. (2016) examined various reticulocyte parameters in individuals with delta-beta thalassemia trait, beta thalassemia trait, and IDA. Their findings indicated that patients with IDA had a higher proportion of immature reticulocytes and a lower absolute reticulocyte count compared to those with beta thalassemia trait. This suggests that, in addition to red blood cell indices, reticulocyte maturity indices may be useful in distinguishing these three clinical conditions.

Conclusion

Our findings suggest that MFR and HFR are high among Sudanese patients with IDA. Thus, the reticulocyte indices may serve as early markers of iron deficiency and anemia.

References

Brugnara C, Schillert B, Moran J (2006). Reticulocyte hemoglobin equivalent (Ret HE) and assessment of iron – deficient states. Clin Lab Haematol. 28(5):303-8.

Buttarello M (2016). Laboratory diagnosis of anaemia: are the old and new red cell parameters useful in classification and treatment, how?. Int J Lab Hematol. 38(Suppl-1): 123-132.

Cappellini MD, Musallam KM, Taher AT (2020). Iron deficiency anaemia revisited. J Intern Med. 287(2): 153-170.

Chandran R,Mohan P (2020). Temporal trends in reticulocyte count with Methylene blue staining: A comparative study to assess reliability of reticulocyte count as a quality control parameter. Int J Clin Diagn Pathol.371-37

Cortellazzi LC, Teixeira SM, Borba R, Gervásio SI, Cintra CS, Grotto HZ (2003). Reticulocyte parameters in hemoglobinophaties and iron deficiency anemia. Rev Bras Hematol Hemoter. 25(2):97-102.

Coyer SM (2005). Anemia: Diagnosis and management. J. Pediatric Health Care. 19(6):380-5.

Habib MA, Black K, Soofi SB, Hussain I, Bhatti Z, Bhutta ZA, et al (2016). Prevalence and predictors of iron deficiency anaemia in children under five years of age in pakistan, a secondary analysis of national nutrition survey Data 2011-2012. PLoS One. 11(5): e0155051.

Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM (2009). Ferritin for the clinician. Blood Rev. 23(3):95-104.

Komal P, Siddhi P (2019). Comparison of automated flowcytometric reticulocyte analysis with manual reticulocyte count. Int J Res Med Sci. 7(10): 3825.

Leal LP, Batista Filho M, Lira PI, Figueiroa JN, Osório MM (2011). Prevalence of anemia and associated factors in children aged 659 months in Pernambuco, Northeastern Brazil. Rev Saude Publica. 45(3): 457-466.

Mawani M, Ali SA, Bano G, Ali SA (2016)p. Iron deficiency anaemia among women of reproductive age, an important public health problem: Situation analysis. Reprod Syst Sex Disord. 5(3): 1000187.

Means RT (2020). Iron deficiency and iron deficiency anaemia: implications and impact in pregnancy, fetal development, and early childhood parameters. Nutrients. 12(2): 447.

Moradabadi A, Khaleghi M, Shahdoost M, Farsinejad A (2019). optimized method for reticulocyte counting: simple, accurate, and comparable to flow cytometry. Iran J Ped Hematol Oncol. 9(1): 17-24.

Thomas C, Thomas L (20002). Biochemical Markers and Hematologic Indices in the diagnosis of Functional Iron Deficiency. Clin Chem. 48(7):1066-76.

Urrechaga E, Borque L, Escanero JF (2011). Erythrocyte and reticulocyte parameters in iron deficiency and thalassemia. J Clin Lab Anal. 25(3): 223-228.