e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2023; 15(9); 22-26

Original Research Article

Determine the Clinical Significance of Immature Reticulocyte Fraction (IRF) and Reticulocyte Maturity Indices in Differential Diagnosis of Anemic Patient: An Observational Study

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Received: 14-03-2023 / Revised: 09-04-2023 / Accepted: 15-06-2023
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Conflict of interest: Nil

Abstract

Aim: The aim of the study was to determine clinical significance of immature reticulocyte fraction (IRF) and reticulocyte maturity indices in differential diagnosis of anemic patient.

Methods: The present study was conducted at the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India for the period of 1year and 200 cases were included in the study. Total of 100 cases, included 48 male and 52 female (40 cases of Iron deficiency anemia, 35 cases of anemia due to chronic kidney disease and 25 cases of thalassemia trait) were studied.

Results: The table showed statistically significant decrease in values of RBC, Hb with retic count was 0.92±0.006, IRF values were also low (0.090±0.060) so as the RETL, RETM, RETH. In thalassemia trait patient, reticulocyte count was high 2.75±0.055 with IRF values were 0.112±0.090, RET L was 89.1±0.090, RET M 7.6±0.060, RET H was 3.5±0.032 observed. The table showed mean retic count of 1.2 with SD of 0.007, and in IRF mean was 0.090 with SD of 0.120 in 35 patients of anemia due to chronic kidney disease. Retic count was low in IDA compare to thalassemia patient (high- hemolytic), IRF values were low in IDA and in CKD patient compare to thalassemia group and regarding reticulocyte indices, statistically significant difference observed for RET H, RET M and RET L between three groups.

Conclusion: Our study showed that with the use of automated fluorescence analyser providing IRF and reticulocyte maturity indices (LFR, MFR and HFR) was quite very useful in the early detection and for the differential diagnosis of iron deficiency anemia, thalassemia and anemia due to chronic kidney disease.

Keywords: immature reticulocyte fraction, reticulocyte indices, anemia

Introduction

Anemia is a condition in which red blood cell numbers, or the hemoglobin concentration inside these cells, is lower than normal. According to the World Health Organization (WHO), the reference value for anemia in males are <13 g/dl and for nonpregnant females are 12 g/dL. [1] The reticulocyte count reflects the erythropoietic activity of bone marrow and is thus useful in both diagnosing anemia and monitoring bone marrow response to therapy. The reticulocytes are young Red Blood Cells (RBCs) that form a reticulum network or granules on exposure to those supravital stains. [2] The existence of the number of reticulocytes in the peripheral blood is a useful clinical indicator of the rate of erythropoiesis. The term Immature Reticulocyte Fraction (IRF) was introduced to indicate the less mature reticulocyte fraction. The IRF represents the proportion of young reticulocytes with the highest RNA content. [3] It is defined as the ratio of immature reticulocytes to the total number of reticulocytes. They are larger, having the greatest light scatter properties due to the highest level of Ribonucleic Acid (RNA). They are one of the newer parameters of automated hematology analyzers and is a sensitive measure of erythropoiesis. [4,5] Pancytopenia is an important clinical and hematological entity worldwide but with varying patterns in clinical presentations. [6,7] Bone marrow aspiration is considered as a primary importance in evaluating and diagnosing the cause of pancytopenia. IRF helps to get a picture about

the marrow erythropoietic activity and also helps in differentiating aplastic anemia from common nutritional deficiency anemia's that presents as pancytopenia. IRF serves as an adjunct to Total Reticulocyte Count (TRC). IRF rises in cases of increased marrow erythropoiesis before the increment of reticulocyte count. Therefore, those cells were found to be the earliest indicator of marrow erythropoietic activity. The IRF value is an early marker for evaluating the regeneration of erythropoiesis. Whereas the IRF percentage increases after only a few hours, the reticulocyte count increases after 2-3 days. If the IRF value does not increase during the treatment of deficiency anemia with erythropoietin or vitamins, this indicates a lack of response to therapy. Together, the IRF value and the reticulocyte count have proven themselves as monitoring parameters for bone marrow and stem cell transplants. [7] The reference range of IRF is 1.6 - 10.5%, for both men and women. The reticulocyte count reflects the erythropoietic activity of bone marrow and is thus useful in both diagnosing anemia and monitoring bone marrow response to therapy. [8] Innovation in automated hematological analyzers, it has become possible to accurately and quickly analyze not only the reticulocyte count but also the reticulocyte cellular index (RCI) and reticulocyte maturity in peripheral blood. [9] Defined by the RNA content of the reticulocytes, reticulocytes are fractioned according to their fluorescence intensity into three maturation stages - LFR (low fluorescence reticulocytes) – mature reticulocytes, MFR (medium-fluorescence reticulocytes) – semi-mature reticulocytes and HFR (high-fluorescence reticulocytes) – immature reticulocytes. IRF is the sum of MFR plus HFR. [10] Thus, the aim of the study was to determine clinical significance of immature reticulocyte fraction (IRF) and reticulocyte maturity indices (LFR, MFR, HFR) in differential diagnosis of anemic patient.

Materials & Methods

The present study was conducted at the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India for the period of 1year and 200 cases were included in the study. Total of 100 cases, included 48 male and 52 female (40 cases of Iron deficiency anemia, 35 cases of anemia due to chronic kidney disease and 25 cases of thalassemia trait) were studied.

Inclusion Criteria

1. All suspected cases of anemia with Hb <13 g/dl in males and <12 g/dl in non-pregnant females are included.

2. All suspected cases of anemia in beta thalassemia trait and chronic kidney disease are included.

3. Patient having anemia of both genders are included.

Exclusion Criteria

1. Children under six months and pregnant females are excluded.

Blood samples from suspected anaemic patients were collected (in K3-EDTA) and proceed further for complete blood count and automated reticulocyte parameters using Horiba Pentra XLR automated analyser within 6 hours of collecting blood. Reticulocyte count is done by fluorescence technology using dye such as thiazole orange [6] that labels RNA and DNA and IRF, LFR (reference range 86.5 - 98.5%), MFR (reference range 1.5 - 11.5 %) and HFR (reference range 0 - 1.4%) values were measured and compared with each group.

Statistical analysis done using SPSS for windows 10.0. Mean and standard values were calculated for the hematological parameters in all cases.

Results

Table 1: Descriptive statistics (Mean and SD) of reticulocyte parameters in Iron deficiency anemia

Parameter	Mean	Standard Deviation
Hb (g/dl)	8.4	±2.020
RBC (million/mm^3)	3.60	±0.840
Retic %	0.92	±0.006
IRF	0.090	±0.060
RET H %	2.4	±0.026
RET M %	6.4	±0.046
RET L %	90.5	±0.065

The table showed statistically significant decrease in values of RBC, Hb with retic count was 0.92±0.006, IRF values were also low (0.090±0.060) so as the RETL, RETM, RETH.

Table 2: Descriptive statistics (Mean and SD) of reticulocyte parameters in thalassemia trait

Parameter	Mean	Standard Deviation
Hb (g/dl)	9.3	±1.850
RBC (million/mm^3)	3.60	±0.920
Retic %	2.75	±0.055
IRF	0.112	±0.090
RET H %	3.5	±0.032
RET M %	7.6	±0.060
RET L %	89.1	±0.090

In thalassemia trait patient, reticulocyte count was high 2.75±0.055 with IRF values were 0.112±0.090, RET L was 89.1±0.090, RET M 7.6±0.060, RET H was 3.5±0.032 observed.

Table 3: Descriptive statistics (Mean and SD) of reticulocyte parameters in CKD

Parameter	Mean	Standard Deviation
Hb (g/dl)	8.6	±1.32
RBC (million/mm^3)	3.70	±0.68
Retic %	1.2	±0.007
IRF	0.090	±0.120
RET H %	2.9	±0.049
RET M %	5.9	±0.072
RET L %	91.9	±0.112

The table showed mean retic count of 1.2 with SD of 0.007, and in IRF mean was 0.090 with SD of 0.120 in 35 patients of anemia due to chronic kidney disease.

Retic count was low in IDA compare to thalassemia patient (high- hemolytic), IRF values were low in IDA and in CKD patient compare to thalassemia group and regarding reticulocyte indices, statistically significant difference observed for RET H, RET M and RET L between three groups.

Discussion

The reticulocytes are young Red Blood Cells (RBCs) that form a reticulum network or granules on exposure to those supravital stains. The reticulum network or granules represent precipitated

rough endoplasmic reticulum with associated polyribosomes.[2] 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. Innovation in automated hematological analyzers, it has become possible to accurately and quickly analyze not only the reticulocyte count but also the reticulocyte cellular index (RCI) and reticulocyte maturity in peripheral blood. [9]

The clinical utility of IRF has been reported in a variety of conditions such as in the diagnosis of anemia (i.e. to determine whether an anemia is hypo-proliferative, ineffective or hemolytic) and its treatment monitoring, transfusion needs, renal transplant engraftment due to Erythropoietin (Epo) production, the detection of hemorrhages or hemolysis, and assessment of the need for RBC transfusion in anemic patient. [11] They also could potentially be useful in the management of neutropenic cancer patients and in the investigation of antimicrobial therapies. [12] Pancytopenia is an important clinical and hematological entity worldwide but with varying patterns in clinical presentations. [13] Bone marrow aspiration is considered as a primary importance in evaluating and diagnosing the cause of pancytopenia. But rather than doing bone marrow aspiration or biopsy, IRF helps to get a picture about the marrow erythropoietic activity. They also help in differentiating aplastic anemia from common nutritional deficiency anemia's that presents as pancytopenia, which is not possible by a reticulocyte count since it is more or less equal in most of the cases. For the diagnosis of various types of anemia, IRF serves as an adjunct to Total Reticulocyte Count (TRC) but is usually not of much help alone in differentiating them all. IRF rises in cases of increased marrow erythropoiesis before the increment of reticulocyte count. Therefore, those cells were found to be the earliest indicator of marrow erythropoietic activity.

Wells et al., has shown that the mean fluorescence intensity of reticulocytes correlated with the serum total iron binding capacity and ferritin concentrations, suggesting that the reticulocyte immaturity is influenced by a patient's iron status. [14] Anemic hypoxia stimulates the release of erythropoietin in the bone marrow, increasing cell proliferation and differentiation. If the reticulocyte concentration increases in the medulla, its maturation will be completed in the blood. [15,16] In case of more severe anemia, the maturation time

of reticulocytes in the marrow decreases, and a greater number of immature reticulocytes are released into the peripheral blood. Which will spend more time in the peripheral blood until they mature into red blood cells. Therefore, the immature reticulocyte count is increases in the peripheral blood. [17]

In thalassemia trait patient, reticulocyte count was high 2.75±0.055 with IRF values were 0.112±0.090, RET L was 89.1±0.090, RET M 7.6±0.060, RET H was 3.5±0.032 observed and findings are consistent with Sedick Q study observation. [18] Anemia in CKD is multi-factorial, but mainly caused by erythropoietin (EPO) production deficiency which is early and frequent complication associated with high morbidity and mortality. IRF is an excellent marker of nearly real-time erythropoietic activity since it represents the proportion of younger reticulocytes in the peripheral blood and rises much earlier than the total number of reticulocytes. [19,20]

The result showed mean retic count of 1.2 with SD of 0.007, and in IRF mean was 0.090 with SD of 0.120 in 35 patients of anemia due to chronic kidney disease. Findings of LFR, MFR and HFR in CKD are consistent with Patricia Scherer study observation. [21] The results showed statistically significant decrease in values of RBC, Hb with retic count was 0.92±0.006, IRF values were also low (0.090±0.060) so as the RETL, RETM, RETH which was consistent with Blessy Mary Thomas study observation. [22]

Conclusion

IRF is a newly routine parameter in hematological analysis, which can give an idea on an early morphological change for bone marrow recovery before other tests to be positive after chemotherapy. The clinical utility of IRF has been reported in a variety of conditions such as in the monitoring of diagnosis of anemia and its treatment, to verify aplastic anemia, and assessment of the need for RBC transfusion in anemic patients. Our study showed that with the use of automated fluorescence analyser providing IRF and reticulocyte maturity indices (LFR, MFR and HFR) was quite very useful in the early detection and for the differential diagnosis of iron deficiency anemia, thalassemia and anemia due to chronic kidney disease.

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