

## Evaluation of Immature Reticulocyte Fraction in Microcytic Hypochromic Anaemias of Paediatric Age Group in Tertiary Care Hospital-a Cross Sectional Study

Zu Afshan Sultana<sup>1</sup>, Fakeha Firdous<sup>2</sup>, Anjani M<sup>3</sup>, Md. Saad Hussain<sup>4</sup>, G J Vani Padmaja<sup>5</sup>

<sup>1</sup>Assistant Professor, Pathology, Niloufer Hospital, Hyderabad.

<sup>2</sup>Assistant Professor, Pathology, Niloufer Hospital, Hyderabad.

<sup>3</sup>Associate Professor, Pathology, Niloufer Hospital, Hyderabad.

<sup>4</sup>Medical Intern, Ayan Medical College, Hyderabad.

<sup>5</sup>Professor, Pathology, Niloufer Hospital, Hyderabad.

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Corresponding Author: Dr Zu Afshan Sultana

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### Abstract

Microcytic hypochromic anemia is characterized by presence of microcytic and hypochromic RBC's on peripheral smear and MCV of less than 80fl. It could be due to iron deficiency anemia or thalassemia or other causes. Differentiation between the thalassemia and non thalassemic microcytosis has important clinical implications, because each has a different causes, pathogenesis, prognosis and treatment.

The assessment of reticulocyte maturation is useful in understanding pathophysiology of anemias.

Immature Reticulocyte Fraction [IRF] represents the proportion of young reticulocytes with highest RNA content. It is a sensitive measure of erythropoiesis. Its clinical utility has been reported in a variety of conditions. It's analysis has been made easy by the use of automated hematology analyzers.

In our study which was carried out in a tertiary pediatric hospital in Telangana, all microcytic hypochromic anemia patients in the age group of new born to 12 years of age were evaluated. IRF is an important parameter which helps in diagnosis and thereby helps in treatment at an early stage itself, thereby decreasing the morbidity in pediatric population.

IRF helps in differentiating etiological causes of anemia like nutritional anemia, hemoglobinopathies and aplastic crisis. It also gives information about the recovery status of anemia cases on treatment.

IRF is a simple, quick, effective and cost effective tool on automated hematology analyzers, which helps in diagnosing anemia cases.

**Keywords:** Immature reticulocyte fraction, IRF, reticulocytes, automated analysers.

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### Introduction

Microcytic Hypochromic anemias can be diagnosed based on the parameters of automated analysers and microscopic examination of peripheral smear which can provide accurate information. The Immature Reticulocyte Fraction [IRF] represents the proportion of young reticulocytes with the highest RNA content. 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 [8].

IRF is the RBCs equivalent of the "left shift" typically associated with neutrophil White Blood Cells (WBCs), providing additional RBC

information that may shorten the time from diagnosis to therapy or therapy itself. Immature reticulocytes normally constitute less than 5% of the total number of reticulocytes.

The normal reference range for IRF is from 3.0%-15.9% in males and 2.3%-13.4% in females.

The manual reticulocyte counts enumerate all RNA stained cells and simply puts together immature and mature reticulocytes. It is also laborious and time-consuming. [1] IRF replaces other Reticulocyte indices like Absolute Reticulocyte Count (ARC) and Reticulocyte Production Index (RPI) that are important to see the degree of erythropoietic activity. A higher proportion of circulating immature reticulocytes (high RNA content) indicates recovering marrow

activity and is quantitated by automated hematology cell analyzers. [1,8] Flow cytometric reticulocyte analysis is more precise and sensitive than manual reticulocyte counting. Besides this, the measured fluorescence intensity allows the quantification of reticulocyte maturity. Automated systems provide a graphic display of the different populations according to the size and amount of RNA. Based on this, the indices are calculated as a percentage of total reticulocytes.

### Clinical Utility of Immature Reticulocyte Fraction (IRF)

The clinical utility of IRF has been reported in a variety of conditions such as :

- In the diagnosis of anaemia (i.e., to determine whether an anaemia is hypo-proliferative, ineffective or haemolytic) .
- Treatment monitoring of Anaemia
- Transfusion needs.
- Renal transplant engraftment due to Erythropoietin (Epo) production
- The detection of haemorrhages or haemolysis, and assessment of the need for RBC transfusion in anaemic patients.
- They also could potentially be useful in the management of neutropenic cancer patients and
- In the investigation of antimicrobial therapies.

**Aim:** This study was done to evaluate IRF in anaemias of paediatric population

#### Materials & Methods:

This prospective study was conducted for a period of One year from May 2022-May 2023 in Department of Pathology in a Tertiary care Paediatric hospital, Hyderabad, under Government of Telangana.

Whole blood samples were collected in EDTA vacutainers from pediatric patients suspected of Anaemia after taking their consent .The Name, age, gender, address etc. were recorded.

**Table 1: Mean IRF in suspected iron deficiency anaemia [ microcytic hypochromic] cases**

Age group	Mean IRF
0-2	25.7
2-6	24.7
6-12	28.34

**Table 2: Mean IRF in cases of hemoglobinopathies**

Age	Mean IRF
0-12 years	27.88

### Discussion

IRF is a relatively new reticulocyte parameter which is available on automated haematology analyzers, which assess the maturity of circulating reticulocytes by quantifying the fraction that stains

### Inclusion Criteria:

- Children with pallor, shortness of breath and easy fatigability
- All cases of microcytic hypochromic anemias diagnosed based on peripheral smear findings and findings on automated hematology analyzers were included.
- Age group- new born to 12 years of age.

### Exclusion Criteria:

History of recent blood transfusion

Diagnosed cases of megaloblastic anemia.

Hematological malignancy cases.

The samples were run in 5 part Automated Analyser XN-1000 and RBC parameters ( RBC Count, Hemoglobin, Hematocrit, MCV, MCH ,MCHC) and Reticulocyte parameters (IRF, LFR, MFR, HFR) were recorded. All the samples were also run in Biorad D10 Analyser for detection of Hemoglobinopathies.

### Results:

A total of 175 samples were collected out of which 15(8.5) cases were diagnosed as Hemoglobiniopathies and rest 155(88.5%) were Iron deficiency anaemia cases.

Out of 175 samples 63 were females (36%) and rest 112 were males (64%).The male to female ratio was 1.77. (Table 1). The parameters were recorded age wise. Age wise 3 groups were formed-0-2 yrs, 2-6 yrs and 6-12 yrs. The lowest Value of Haemoglobin detected was 2.4g/dl and highest value was 12.8gm/dl.

Lowest value of RBC count, MCV, MCH detected were  $0.85 \times 10^6$ , 47.7fl & 10.0pcgm respectively.

Lowest value of IRF was found to be 4.6 and highest value of IRF was 49.

strongest for RNA.IRF will appear in the reticulocyte section of the CBP report below RET, it is calculated ratio of newly released reticulocytes to total reticulocytes. Increased IRF generally indicate an adequate erythroid response to anemia. Normal

reference range in males-3-15.9%,females 2.3 to 13.4%. [2]The most common causes of microcytic hypochromic anemia in Pediatric patients are iron deficiency anemia and thalassemia. IRF has an important role in differentiating both.

The manual reticulocyte counts enumerate all RNA stained cells and simply puts together immature and mature reticulocytes. It is also laborious and time consuming. Assessment of reticulocyte maturity is based on the intensity of either fluorescence or light scattering/absorbance, which depends on RNA content. Different populations are discriminated by a software-based algorithm that usually clusters reticulocytes into 3 areas according to stain intensity. The fluorescence intensity of the entire reticulocyte population was initially reported as the reticulocyte maturation index or the mean fluorescent index. Reticulocytes have now been grouped into the Low Fluorescent Region (LFR), Middle Fluorescent Region (MFR) or High Fluorescent Region (HFR) corresponding to the lower, middle and higher RNA content, respectively. The percentage of reticulocyte is given as the sum of LFR, MFR and HFR. [3,4,5] IRF measures the MFR and HFR populations and is more reproducible than the HFR. Immature fractions have larger amounts of RNA than mature reticulocytes. Thus, the uses of fluorescent probes that label the RNA permit the differentiation and quantification of the IRF.

Choi and son evaluated the IRF of 149 patients and the means of IRF were found to be 1.13% for healthy individuals and 2.10% for patients with iron deficiency anemia. [6] thus our study like the choi and son study revealed that IRF started to increase as serum iron levels decreased reaching its peak when the patients had an evident iron deficiency.

Butthep etal [7,8] evaluated reticulocyte maturity in 141 individuals, among them patients with thalassemia and healthy individuals,IRF value showed significant increase like in our study.

**Conclusion:**It is a very sensitive marker of marrow erythropoietic activity.

IRF quantifies the fraction of circulating reticulocytes that are least mature and just exited the bone marrow. This parameter could help in diagnosing cases of haemolytic anemia, nutritional anemias, aplastic crisis and recovery status. IRF predicts reticulocyte recovery in patients. Thus, IRF

gives a basic idea about marrow erythropoietic activity and its response to drugs and therapy. It is thus a simple, quick, cost effective, reproducible and reliable tool on the automated hematology analyser.

**Consent:** Informed written consent from parents/guardians.

**Ethical Clearance:** Taken from college ethical committee.

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