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Clinical significance of immature reticulocyte fraction (IRF) and reticulocyte maturity indices in differential diagnosis of anemia

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Abstract
Background: Anemia is most common public health problems in developing countries. It is important to know the cause of anemia to treat the patients. The Immature reticulocyte fraction (IRF) value is an early marker for evaluating the regeneration of erythropoiesis. In addition to conventional reticulocyte measurement, the fluorescence method allows the classification of reticulocytes into three maturation stages – LFR, MFR and HFR.
Aims: To determine clinical significance of immature reticulocyte fraction (IRF) and reticulocyte maturity indices (LFR, MFR, HFR) in differential diagnosis of anemic patients.
Material and Method: IRF and other reticulocyte parameters were measured by automated blood cell analyzer, Horiba Pentra XLR using fluorescence marker that labels RNA and DNA (such as thiazole orange or polymethines), in 124 patients with anemia. The anemia group consist of 50 iron deficiency anemia (IDA) patients, 29 cases of thalassemia trait patients and 45 patients with anemia due to chromic kidney diseases (CKD). In this retrospective and prospective type study, the values of IRF and other parameters were analysed and compared in each group for the duration of one year.
Result: Total of 124 cases were evaluated with median age of age of 32.8 years showed that mean value of IRF was low in IDA (0.096±0.058) and in CKD (0.092±0.111) compare to thalassemia (0.116±0.094). The mean values of MFR and HFR were also found to be significantly lower in IDA group and in CKD when compared to the thalassemic group. Values of LFR were higher in IDA and in CKD compare to thalassemia.
Conclusions: IRF assesses reticulocyte maturation by the intensity of the staining that reflects the mRNA content. The evaluation of IRF and reticulocyte maturity indices by automated cell counter analyzer seemed to be accurate and clinically useful for the early diagnosis of anemia and the differentiation of IDA and CKD from thalassemia.

Keywords: IRF, iron deficiency anemia, thalassemia, chronic kidney disease, reticulocytes

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 non-pregnant females are 12 g/dL. Approximately 30% of the world population is estimated to have anemia, with most of these cases being caused by iron deficiency [1] though haemoglobinopathies and infectious diseases are also the important causes.

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 [2]. 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 anaemias 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 [3]. The reference range [4] of IRF is 1.6 - 10.5%, for both men and women.

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 [5].

Materials and Methods

- **Place of Study:** The present study was carried at the department of pathology in CCL and OPD 18 section of G.G.G.H hospital, Jamnagar.
- **Type of Study:** Prospective and Retrospective Study.
- **Duration of study:** 1year
- **Sample size:** Total 124 cases.

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.

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.

Results

The present study was conducted from December 2021 to December 2022 in the Department of Pathology, at tertiary care hospital, total of 124 case, includes 61 male and 63 female (50 cases of Iron deficiency anemia, 45 cases of anemia due to chronic kidney disease and 29 cases of thalassemia trait) were studied with median age of 32.8 years (02-75 yrs) and following observations were made.

Out of 50 cases of iron deficiency anemia, majority patients were female. It shows statistically significant decrease in values of RBC, Hb with retic count was 0.95±0.004, IRF values were also low (0.096±0.058) so as the RETL, RETM, RETH (Table 1).

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

Parameter	Mean	Standard Deviation
Hb (g/dl)	7.4	±2.015
RBC (million/mm^3)	3.57	±0.860
Retic %	0.95	±0.004
IRF	0.096	±0.058
RET H %	2.8	±0.028
RET M %	6.7	±0.047
RET L %	90.3	±0.060

Hb: Hemoglobin; RBC: Red blood cell count; Retic%: Reticulocyte count; IRF: Immature reticulocyte fraction; RET H: high-fluorescence reticulocytes; RET M: medium-fluorescence reticulocytes; RET L: low-fluorescence reticulocytes.

In thalassemia trait patient, reticulocyte count was high 2.89±0.045 with IRF values were 0.116±0.094, RET L was 88.3±0.094, RET M 7.7±0.065, RET H was 3.8±0.036 observed (Table 2).

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

Parameter	Mean	Standard Deviation
Hb (g/dl)	8.4	±1.892
RBC (million/mm^3)	3.58	±0.912
Retic %	2.89	±0.045
IRF	0.116	±0.094
RET H %	3.8	±0.036
RET M %	7.7	±0.065
RET L %	88.3	±0.094

Table 3 shows mean retic count of 1.1 with SD of 0.005, and in IRF mean was 0.092 with SD of 0.111 in 45 patients of anemia due to chronic kidney disease.

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

Parameter	Mean	Standard Deviation
Hb (g/dl)	8.3	±1.31
RBC (million/mm^3)	3.63	±0.62
Retic %	1.1	±0.005
IRF	0.092	±0.111
RET H %	2.7	±0.044
RET M %	5.8	±0.075
RET L %	91.5	±0.114

Table 4 shows comparison of IRF, RETH, RETM, RETL and other hematological parameters between IDA, thalassemia and CKD patients (Values are mean ± standard deviation). 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.

Table 4: Comparison of Immature reticulocyte fraction and other reticulocyte parameters between Iron deficiency anemia, thalassemia trait and in CKD patients

Parameter	IDA (n = 50)	Thalassemia trait (n = 29)	CKD (n = 45)
Hb (g/dl)	7.4±2.015	8.4±1.892	8.3±1.31
RBC (million/mm^3)	3.57±0.860	3.58±0.912	3.63±0.62
Retic %	0.95±0.004	2.89±0.045	1.1±0.005
IRF	0.096±0.058	0.116±0.094	0.092±0.111
RET H %	2.8±0.028	3.8±0.036	2.7±0.044
RET M %	6.7±0.047	7.7±0.065	5.8±0.075
RET L %	90.3±0.06	88.3±0.094	91.5±0.114

Discussion

The present study was aimed at evaluating the utility of IRF and reticulocyte maturity indices in the differential diagnosis of IDA, thalassemia and CKD which shows immaturity of reticulocytes and the bone marrow activity through

fluorescence intensity [7].

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 [8]. 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 [7, 9]. 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 [10]. So, we believe that in the presence of iron deficiency the indices related to the immaturity of reticulocytes increase, thus demonstrating a deficiency of the raw material required for the formation of Hb. These results are consistent with other studies since they also presented elevations of MFR and HFR in iron deficiency anemia [10, 11, 7].

Microcytic anemia in the case of thalassemia results from impaired globin chain synthesis and decreased Hb synthesis [12]. The reticulocyte count in thalassemia carriers correlated with the degree of ineffective erythropoiesis, despite that it is accelerated [13]. The evaluation of reticulocyte maturation might be useful for understanding the different pathophysiology and helpful in differential diagnosis of these anemias [14]. In IDA, the erythroid expansion leads to an enhanced immature reticulocyte release from bone marrow to compensate the anemic status. The moderate increase in IRF in thalassemia carriers, in spite of the chronic increase in erythropoiesis, reflects that it is severely impaired. This can be explained by the fact that erythropoietin levels in β thalassemia minor are significantly lower than in IDA with the same degree of anemia [15]. Our study showed that mean IRF values were higher (0.116) in thalassemia compare to mean IRF values in IDA (0.096), findings are consistent with Sedick Q [16] study observation.

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 [17, 18]. Out study shows that Mean value of Hb was low in relation to IRF indicated towards ineffective erythropoiesis. Findings of LFR, MFR and HFR in CKD are consistent with Patricia Scherer [19] study observation.

In case of iron deficiency anemia (Mean IRF 0.096) due to nonavailability of iron, in case of chronic kidney disease anemia (Mean IRF 0.092) due to lack of erythropoietin and in thalassemia (Mean IRF 0.116) due to ineffective erythropoiesis there were low values of IRF were observed which is consistent with Blessy Mary Thomas [20], study observation.

Conclusion

Out 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.

Conflict of Interest

Not available

Financial Support and sponsorship

Not available

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