ORIGINAL RESEARCH

Evaluate the clinical relevance of the immature reticulocyte fraction (IRF) and reticulocyte maturity indices in distinguishing between various causes of anaemia in patients

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ABSTRACT

Aim: The aim of the study is to evaluate the clinical relevance of the immature reticulocyte fraction (IRF) and reticulocyte maturity indices in distinguishing between various causes of anaemia in patients.

Methods: The present study is conducted in the Department of Pathology for a period of one year and 100 cases were included in the study.

Results: The result showed mean retic count of 1.3 with SD of 0.007, and in IRF mean was 0.091 with SD of 0.120. Retic count was low in IDA compared to thalassemia patient (high- hemolytic), IRF values were low in IDA and in CKD patient compared to thalassemia group and regarding reticulocyte indices, statistically significant difference was 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.

Key words: Immature reticulocyte fraction, reticulocyte indices, anemia

INTRODUCTION

Anemia is a condition in which red blood cell counts, or the hemoglobin content within these cells, is lower than normal. According to the World Health Organization (WHO), the reference value for anemia in men are <13 g/dl and for nonpregnant females are <12 g/dL.[1] The reticulocyte count represents the erythropoietic activity of bone marrow and is therefore valuable in both diagnosing anemia and evaluating bone marrow response to medication. The reticulocytes are juvenile Red Blood Cells (RBCs) that develop a reticulum network or granules on exposure to such supravital stains.[2]

The blood count is the initial laboratory test used to identify and guide the investigation of anaemia.[3] According to the World Health Organization (WHO), anemia is a condition in which hemoglobin concentration in the blood is below the reference limits, being <13 g/dL and <12 g/dL for men and women, respectively.[4] After the diagnosis of anemia, erythrocyte characteristics such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) facilitate a search of the likely causative pathways. In addition, they provide the foundation for the morphological categorization encompassing normochromic and normocytic, microcytic and hypochromic, and macrocytic anemias.[5] Considering that microcytic anemia is the most often encountered anemia in general medical practice, notably in children and adolescents, improving the capabilities of laboratory tests for their differential diagnosis is of considerable relevance.[6] The kinds of anemia with these physical features

include iron deficiency anemia, thalassemias, anemia of chronic illness and sideroblastic anemia.[7] Erythrocyte characteristics, and both reticulocyte count and indices are also regarded highly valuable tools in the differential diagnosis of anemia and its therapy.[8]

Innovation in automated hematological analyzers, it has become feasible to correctly and rapidly examine 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. The IRF is the aggregate of the MFR and HFR.[10] The aim of the research was to evaluate the clinical relevance of the immature reticulocyte fraction (IRF) and reticulocyte maturity indices in distinguishing between various causes of anaemia in patients.

MATERIALS & METHODS

The present study is conducted in the Department of Pathology for a period of one year and 100 cases were included in the study.

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.

METHDOLOGY

The Horiba Pentra XLR automated analyzer was used within 6 hours of blood collection to conduct a full blood count and automated reticulocyte parameters on individuals who were suspected of having anaemia. The blood samples were collected in K3-EDTA. We evaluated and compared the levels of IRF, LFR (reference range 86.5-98.5%), MFR (reference range 1.5-11.5%), and HFR (reference range 0-1.4%) with each group using fluorescence technology that employs dyes such thiazole orange.[6]

STATISTICAL ANALYSIS

Windows version of SPSS, version 20.0, was used for the statistical analysis.

RESULTS

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

Parameter	Mean	Standard Deviation
Hb (g/dl)	8.1	±2.12
RBC (million/mm^3)	3.2	±0.74
Retic %	0.89	±0.017
IRF	0.09	±0.032
RET H %	2.2	±0.017
RET M %	6.1	±0.027
RET L %	90.1	±0.016

The result showed statistically significant decrease in values of RBC, Hb with retic count was (0.89±0.007), IRF values were also low (0.09±0.032) so as the RETL, RETM, RETH.

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

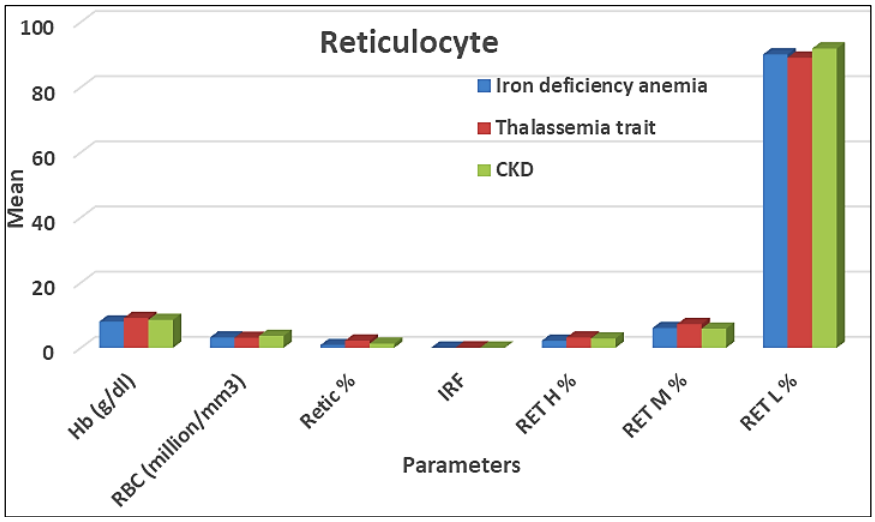
Parameter	Mean	Standard Deviation
Hb (g/dl)	9.2	±1.852
RBC (million/mm^3)	3.1	±0.922
Retic %	2.26	±0.056
IRF	0.12	±0.092
RET H %	3.2	±0.024
RET M %	7.3	±0.052
RET L %	89.1	±0.082

In thalassemia trait patient, reticulocyte count was high 2.26±0.034 with IRF values were 0.12±0.092, RET L was 89.1±0.082, RET M 7.3±0.052, RET H was 3.2±0.024 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 %	1.3	±0.007
IRF	0.091	±0.120
RET H %	2.9	±0.049
RET M %	5.9	±0.072
RET L %	91.9	±0.112

The result showed mean retic count of 1.3 with SD of 0.007, and in IRF mean was 0.091 with SD of 0.120. 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.



Graph 1: reticulocyte parameters in all three conditions

DISCUSSION

Reticulocytes are immature Red Blood Cells (RBCs) that generate a network of granules when exposed to particular stains that may be employed while the cells are still living. After polyribosomes and rough endoplasmic reticulum precipitate, reticulum networks or granules develop.[2] The reticulocyte count acts as an index of the bone marrow's erythropoietic activity, making it important for diagnosing anaemias and following the bone marrow's response to therapy. Advancements in automated haematological analyzers have permitted accurate and quick detection of not only the reticulocyte count, but also the reticulocyte cellular index (RCI) and reticulocyte maturity in peripheral blood.[9]

The clinical usefulness of IRF has been documented in various conditions, including the diagnosis of anaemia (specifically to determine if it is hypo-proliferative, ineffective, or hemolytic) and its monitoring during treatment, assessment of transfusion requirements, evaluation of renal transplant engraftment through Erythropoietin (Epo) production, detection of haemorrhages or hemolysis, and determination of the need for red blood cell (RBC) transfusion in anaemic patients.[11] They may also help treat cancer patients with low neutrophil counts and study antimicrobial treatments.[12] Worldwide, pancytopenia is a serious clinical and haematological disorder with different clinical presentations.[13] To determine pancytopenia's cause, bone marrow aspiration is essential. Instead of aspirating or biopsying bone marrow, IRF measures erythropoietic activity. Additionally, they serve in separating aplastic anaemia from nutritional deficiency anaemia that appears as pancytopenia. This distinction cannot be performed with a reticulocyte count, as it stays rather stable in the majority of situations. For the diagnosis of various kinds of anemia, IRF acts as an addition to Total Reticulocyte Count (TRC) but is typically not of much use alone in distinguishing them all. IRF increases in situations of enhanced marrow erythropoiesis before the elevation of reticulocyte count. Therefore, those cells were determined to be the early sign of marrow erythropoietic activity.

Anaemic hypoxia promotes the release of erythropoietin in the bone marrow, which increases the proliferation and specialisation of cells. If medulla reticulocyte concentration rises, blood maturation completes.[14,15] In severe anaemia, reticulocyte maturation time in the marrow decreases, releasing more immature reticulocytes into the peripheral blood.

They will stay in the peripheral blood longer until they mature into red blood cells. Thus, peripheral blood immature reticulocytes increase.[16]

The study found a significant decrease in RBC, Hb, IRF, RETL, RETM, and RETH values, with a retic count and IRF values. In thalassemia trait patient, reticulocyte count was high with IRF values were 0.113±0.092, RET L was 89.2±0.092, RET M 7.3±0.062, RET H was 3.6±0.034 observed.

CKD anaemia is multifactorial, but erythropoietin (EPO) production deficiency is the most common and deadly complication. IRF represents the proportion of younger reticulocytes in the peripheral blood and rises much earlier than the total number, making it a good indicator of nearly real-time erythropoietic activity.[17,18]

The mean retic count was 1.3 with SD of 0.007, while IRF was 0.091 with SD of 0.120. Retic count was low in IDA compared to thalassemia patient (high-hemolytic), IRF values were low in IDA and CKD patients, and RET H, M, and L were statistically different across three groups. CKD LFR, MFR, and HFR findings match Patricia Scherer's research.[19]

CONCLUSION

The newly proposed haematological parameter IRF may identify early morphological changes in bone marrow healing before other tests reveal good outcomes following chemotherapy. IRF is used to monitor anaemia diagnosis and therapy, confirm aplastic anaemia, and determine RBC transfusion needs in anaemic patients. We found that the automated fluorescence analyzer, which gives IRF and reticulocyte maturity indices (LFR, MFR, and HFR), was successful in detecting and distinguishing iron deficiency anaemia, thalassemia and chronic kidney disease-related anaemia.

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