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ROLE OF IMMATURE RETICULOCYTE FRACTION IN CLASSIFICATION OF HYPOREGENERATIVE ANEMIAS

Dr. Jayanti Nayak[1] and Dr. Pramita Sahu[2]*

[1]Associate Professor, Department of Pathology, MKCG Medical & Hospital, Berhampur, Odisha.
[2]Assistant Professor, Department of Pathology, MKCG Medical College & Hospital, Berhampur, Odisha.

***Corresponding Author: Dr. Pramita Sahu**
Assistant Professor, Department of Pathology, MKCG Medical College & Hospital, Berhampur, Odisha.

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ABSTRACT
Anaemia is a global health problem. India has a high prevalence of anaemia. Anemia is categorized to hypo-regenerative anaemias and regenerative anaemias based on the absolute reticulocyte count. The immature reticulocyte fraction (IRF) gives a basic idea about the marrow erythropoietic activity and its response to drugs and therapy at an early stage thereby useful for monitoring therapy by the physicians without seeing the marrow. In our study 312 cases of anemia were studied. The most common cause of hypo-regenerative anemia in our study was found to be megaloblastic anemia with 93 (29.8%) followed by iron deficiency anemia anemia (28.2%). There was no significant difference in Hb and reticulocyte percentages across the anemia groups, $p>0.05$, but the present study observed a weak but significant correlation while comparing IRF with RT%, ARC and Hb ($p<0.05$). The conclusion of the present study was, considering the ARC and IRF values along with peripheral smear examination give further clues to the differential diagnosis of anaemia and it can also aid in differentiation of aplastic anaemia from other causes of pancytopenia as true aplastic anaemia cases have zero IRF along with low reticulocyte count.

KEYWORDS: Pancytopenia, Immature Reticulocyte Fraction, Aplastic anemia.

INTRODUCTION
Anaemia is a global health problem. 1.62 billion people, globally, are anemic, which corresponds to 24.8% of the population, out of which women in reproductive age group are the major contributors (42%).[1] India has a high prevalence of anaemia with the NFHS-3(National Family Health Survey) indicating the prevalence of anaemia to be 70-80% in children, 55% in women, 70% in pregnant women and 24% in adult men. It has been estimated that about 20-40% of maternal deaths in India are due to anaemia.[2]

Anaemia is categorized to hypo-regenerative anaemias and regenerative anaemias based on the absolute reticulocyte count[3] and also to microcytic hypochromic type, normocytic type and macrocytic type based on the mean corpuscular volume.[4] The hypo-regenerative anemias include Aplastic anemia, Megaloblastic anemia, Myelodysplastic syndrome, marrow infiltration/ fibrosis and erythropoietin underproduction.

Most of the physicians use only reticulocyte percentage and absolute reticulocyte count in evaluating anemias. We attempted to emphasize the importance of Immature Reticulocyte Fraction (IRF) in diagnosis and classification of hyporegenerative anemias.

MATERIAL AND METHODS
A prospective study was done in our department from November 2014 to October 2016. All patients with anemia, defined as hemoglobin value less than 10g/dl in women, 12g/dl in men and 11g/dl in children, were evaluated with detailed clinical history and clinical examination. Children less than 5 years of age, those receiving treatment for anemia, including blood transfusion, and patients receiving chemotherapy were excluded from the study.

Two ml of EDTA blood sample were collected in vacutainers. Complete haemogram with reticulocyte indices were done with automated hematology analyzer Sysmex XT- 2000i and simultaneous peripheral blood smear examination were done. All the patients with absolute reticulocyte count (ARC) < $50x10^9$/L were diagnosed as having hyporegenerative anemia and included in the study group. 50 persons with hemoglobin values more than 13 g/dl in men and 12 g/dl in women and children were taken as control. Data were evaluated by standard statistical methods.

RESULT
A total of 312 cases of hypo-regenerative anemias were studied. Patients presented with different age groups with

most common affected age group of 16-30 years. Males were more affected than females. Complete blood count with reticulocyte indices and peripheral smear examination were performed in all the cases. Bone marrow aspiration was done in selected cases where indicated. The most common cause of hypo-regenerative anemia was found to be megaloblastic anemia with 93 (29.8%) followed by iron deficiency anemia anemia (28.2%). Other less common causes were anemia associated with leukemia/ hematological malignancy, aplastic anemia, idiopathic myelosuppression, anemia of chronic disease, myelodysplastic syndrome and aplastic crisis in sickle cell anemia. The reticulocyte percentage, absolute reticulocyte count and IRF were compared in all the cases and the results were correlated and tabulated (Table-1).

Table 1: Mean of hematological parameters in hypo-regenerative anemias.

Sl. No.	Causes of anaemia	Hbgm/dl	RT%	ARC x 10^3/µl	IRF %
1	Megaloblastic An.	5.4	1.32	44.8	8.6
2	Iron Def Anemia	6.7	1.56	43.1	19.4
3	An Asso with Leukemia/ Hematological malig	6.3	2.07	47.9	15.7
4	Aplastic Anemia	4.7	0.46	10.4	0
5	Idiopathic Myelosuppression	7.1	2.35	48.6	14.5
6	An of Chr Disease	9.2	0.63	37.2	9.3
7	Myelodysplastic Syndrome	6.8	0.84	19.2	13.4
8	Aplastic crisis in Sickle Cell Anemia	4.9	0.29	8.8	0
9	Control	12.4	0.34	56.1	5.2

There is no significant difference in Hb and reticulocyte percentages across the anemia groups, $p>0.05$, but the present study observed a weak but significant correlation while comparing IRF with RT%, ARC and Hb ($p<0.05$).

DISCUSSION

Anemia is a major health concern in India as well as worldwide which imparts great loss in terms of one's productivity and health and as a whole in terms of economy of a nation. A total number of 312 patients were included in the study and 50 healthy individuals with normal RBC parameters were taken as control.

The reticulocyte count is clinically important for evaluating the erythropoietic activity of bone marrow and for diagnosis of anemia. Flow cytometry using a fluorescent stain that binds to ribosomal RNA produces more reliable results than the manual method, and provides information about reticulocyte immaturity. The number of immature reticulocytes in peripheral blood reflects an increase in erythropoiesis and in red blood cell turnover or a hyporesponsive marrow.[5]

The difference in staining makes possible the identification of the youngest highly fluorescent reticulocytes from the more mature low fluorescent reticulocytes based on which they are divided into Low fluorescence reticulocyte (LFR), Medium fluorescence reticulocyte (MFR) and High fluorescence reticulocyte (HFR).[6] The sum of Medium fluorescence ratio and High fluorescence ratio is given as the Immature reticulocyte fraction (IRF). Immature reticulocytes normally constitute less than five percent (5%) of the total number of reticulocytes.[7]

The present study observed Megaloblastic anemia as the most prevalent hyporegenerative anemia, which accounts for 29.8% of cases. This is in contrast to the observation in most of the studies, including NFHS 2008, that iron deficiency anemia is the most prevalent one. The discrepancy noted in the present study may be attributed to the less number of cases included in the study group as well as the fact that, ours being a tertiary care center, not all cases of iron deficiency anemia were sent to us for detailed investigation.

The reticulocyte count and maturity in 50 normal healthy volunteers, who had normal hemoglobin and red cell parameters as per standard reference values for age and sex, were analyzed; we found the mean for ARC in healthy controls to be 56.1; and IRF to be 5.2. These values were in concordance with other studies.[8,9] We found significant difference in mean value of ARC and IRF when comparing healthy controls to the study groups ($p< 0.05$). Besides this, significant difference in terms of ARC and IRF among the various etiological groups ($p<0.05$) was noted.

Based on the IRF, we divided the hyporegenerative anemias into three groups (Table 2) - First group having zero IRF, second group with low (<10%) IRF and third group with IRF being more than 10%.

Table 2: Classification of hyporegenerative anemias based on IRF.

GROUP	CRITERIA	ETIOLOGY
I	IRF=0	Aplastic anemia, Aplastic crisis in Sickle cell disease
II	IRF<10%	Megaloblastic anemia, Anemia of chronic disease
III	IRF>10%	IDA, Anemia associated with hematological malignancies, Idiopathic myelosuppression, MDS

This categorization in the present study using IRF can be useful not only for differentiating aplastic anemia (IRF=0) from other causes of pancytopenia but also in the overall differential diagnosis of hyporegenerative anemias. Davis et al[10] stated that true aplastic patients or transient state of erythroid aplasia (as seen in early stages of bone marrow transplantation or drug induced aplasia) demonstrate subnormal IRF and reticulocyte count. In contrast, patients in the early stages of an erythropoietic response (as in bone marrow engraftment or during recovery from myelotoxic drug insult) would demonstrate high IRF along with subnormal reticulocyte count. Sindhu et al[11] used ARC and IRF to categorize pancytopenia in to 4 categories and, found them to be useful in differentiating aplastic anemia from megaloblastic anemia. However, our study differs from the study of Watanabe et al[12] who found high levels of IRF in cases of aplastic anemia.

CONCLUSION

IRF and reticulocyte count provide an idea about the marrow erythropoietic activity and aid in subclassifying anaemia into hyporegenerative and regenerative marrow subtype. Considering the ARC and IRF values along with peripheral smear examination give further clues to the differential diagnosis of anaemia. It can also aid in differentiation of aplastic anaemia from other causes of pancytopenia as true aplastic anaemia cases have zero IRF along with low reticulocyte count.

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