

Reticulocyte Study in Different Neoplastic and Non- Neoplastic Conditions of Haematology

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Abstract

Introduction: Reticulocytes are immature red blood cells. Reticulocytes occupy an intermediate position between nucleated RBC and mature RBC. They are called reticulocytes because of reticular network of ribosomal RNA. Reticulocytes undergo removal of RNA on passing through spleen on 1st day and are important indicator of RBC production.

Aims: To study the significance of reticulocyte count in preliminary evaluation of various haematological conditions in patients who underwent bone marrow examination.

Material and Methods: The present study was a descriptive study. This Study was conducted 18 months (from March 2021 to September 2022) at department of Pathology for haematological evaluation in D.M.C.H. Total 100 patients were included in this study.

Result: In our study, 9 (9.0%) patients had ALL Diagnosis, 7 (7.0%) patients had AML, 6 (6.0%) patients had Drug Induced hemolytic anemia, 1 (1.0%) patients had Essential Thrombocythemia, 30 (30.0%) patients had IDA, 2 (2.0%) patients had IDA on Treatment, 3 (3.0%) patients had MAHA, 8 (8.0%) patients had Malaria, 18 (18.0%) patients had Megaloblastic Anemia, 2 (2.0%) patients had Megaloblastic Anemia on treatment, 5 (5.0%) patients had Thalassemia and 9 (9.0%) patients had Visceral Leishmaniasis. The value of z is 5.6662. The value of p is < 0.00001. The result is significant at p < 0.05.

Conclusion: We conclude that, higher number of patients had IDA which was statistically significant. All these clinical and histo-morphological parameters and advanced newer diagnostic modalities like immunohistochemistry, morphometric analysis can help to early diagnosis and to plan the line of treatment and also have prognostic significance.

Keywords: IRF, MRV, Pancytopenia and Atypical Features.

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Introduction

Reticulocytes are immature red blood cells. Reticulocytes occupy an intermediate position between nucleated RBC and mature RBC. They are called reticulocytes because of reticular network of ribosomal RNA. Reticulocytes undergo removal of

RNA on passing through spleen on 1st day and are important indicator of RBC production. As its life span is short i.e. 1 day with normal haematocrit and as high as 2.5 days when haematocrit is below 20, it accounts for latest status of marrow

activity. They can be detected in peripheral blood smear by staining with supravital stains like Brilliant Cresyl Blue, but newer cell counter can give accurate reticulocyte count based on the fact that various dyes and fluorochrome combines with RNA of reticulocytes.

Normal Reticulocyte Percentage is 0.5% to 1.5%

Absolute Reticulocyte Count (ARC) = Reticulocyte % x RBC Count

Normal ARC in adults & children = 25,000 to 75000/microlitre

Neutrophil count, as their life span is small is very useful to assess Bone Marrow activity at the earliest. Reticulocytes & particularly together with immature reticulocytes fraction (IRF) is also extremely sensitive parameter to assess bone marrow activity at given time.

The classification of anaemia on the basis of reticulocyte count has been advocated since long — hypo proliferative/hyper proliferative.

A modern 6 part cell counter make reticulocytes study very handy and convenient. It gives

- i) Reticulocyte Percentage (%)
- ii) Absolute Reticulocyte Count (ARC)
- iii) Immature Reticulocyte Fraction (IRF)

Study of haematological cases with proper history, proper general & systemic examination particularly organomegaly, proper correlation & consideration of all RBC indices together with platelet & leucocytes & parenthetically careful screening of peripheral blood smear in conjunction with reticulocytes study is expected to deliver very useful insight in haematological & non-haematological conditions.

Hematology analyzers can count reticulocytes and quantify their maturity. Automated evaluation helps in providing accurate information about the age distribution of the reticulocyte population, eliminates any subjective technical

variability and imprecise counting of manual evaluation, and is cost-efficient for the evaluation of a large number of samples. [1] Nowadays, newer reticulocyte indices like reticulocyte count and percentage, mean reticulocyte volume (MRV), and immature reticulocyte fraction (IRF) by automated hematology analyzers help us to get an insight into the marrow erythropoietic activity and are useful in the evaluation of pancytopenia.

Reticulocyte percentage (retic %) indicates the rapidity of red blood cell turnover. Normal retic % is 1%–2% and represents the daily replacement of 0.8%–1% of the circulating red blood cells. [2]

The term IRF was proposed to indicate the least mature fraction of reticulocytes. [3] It is the ratio of immature reticulocytes to the total number of reticulocytes. This parameter is an early and sensitive index of erythropoietic activity of the bone marrow. The best clinical use, in the classification of anemias based on the evaluation of erythropoietic response, can be obtained. The IRF result is reported along with the reticulocyte count (both percentage and absolute value). Its reference value is 0.11–0.38. [4]

MRV is 24% higher than the mean cell volume (MCV) of mature erythrocytes. The MRV multiplied by the number of reticulocytes gives the “hematocrit” value of the reticulocytes. It helps in the monitoring of treatment response and also is a sensitive marker of erythropoiesis. [5]

So far, megaloblastic anemias and dual deficiency anemias (DDA) are diagnosed by bone marrow aspiration which is invasive, or by biochemical parameters which are costly, and which may not be reliable since values are vulnerable to change with a good diet in the hospital. [6] Reticulocyte indices can play a pivotal role in identifying the cause of different neoplastic and non- neoplastic conditions of haematology avoiding invasive procedures or expensive testing.

Materials and Method

The study was an institution based cross sectional descriptive study over a period of 18 months from March 2021 to September 2022 with sample size of 100.

Inclusion Criteria:

All indoor patients of Medicine and Paediatrics department and patients coming to Pathology department for haematological evaluation in D.M.C.H.

Exclusion Criteria:

All pregnant and breastfeeding women and patients who are on any chemotherapy or radiotherapy.

Following Lab Tests Will Studied:-

- 1) CBC with RBC indices
- 2) Absolute Reticulocyte Count
- 3) Peripheral Blood Smear
- 4) Bone Marrow Aspiration In selected Cases
- 5) Serum vitamins B12
- 6) Serum Folate
- 7) Any other ancillary test as required

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Result and Discussion

The present study was a descriptive study. This Study was conducted 18 months (from March 2021 to September 2022) at department for haematological evaluation in D.M.C.H. Total 100 patients were included in this study.

Kaur M et al [7] (2014) found that the bone marrow is frequently involved in variety of cases presenting with hematological and non-hematological disorders, which are diagnosed by two separate but interrelated

techniques such as bone marrow aspiration (BMA) and bone marrow biopsy (BMB). A wide age range from 4 years to 74 years.

Sindhiya CV et al [8] (2022) examined that pancytopenia is a condition in which red blood cells, white blood cells, and platelets are lower than the normal range. The age ranged from 45days to 85years had male preponderance with generalized weakness, easy fatiguability, and fever being common complaints. The patients had a male to female sex ratio of 1.6:1.

In my study, out of 100 patients most of the patients were 21 to 30 years old [21 (21.0%)]. Which was statistically significant ($p=.00438$), ($z=2.853$).

Kaur M et al [7] (2014) found that the bone marrow is frequently involved in variety of cases presenting with hematological and non-hematological disorders, which are diagnosed by two separate but interrelated techniques such as bone marrow aspiration (BMA) and bone marrow biopsy (BMB).

Present study showed that, higher number of the patients were male [64 (64.0%)]. Male: Female ratio was =1.8:1. Which was statistically significant ($p=.00008$), ($z=3.9598$)

I found that, most number of patients had Microcytic Hypochromic [30 (30.0%)]. It was statistically significant ($p<.00001$), ($z=5.6662$)

Beham-Schmid C et al [9] (2020) found that the normal red blood cell count is kept by the balance between erythrocyte production and erythrocyte destruction. The regulation of progenitor cells to the erythroid lineage and their maturation are controlled by growth factors, cytokines and, very important, by the bone marrow microenvironment signaling directing nuclear transcription factors and gene expression.

Suárez EU et al [10] (2022) observed that an 80-years-old woman presented with 6-weeks history of fatigue and shortness of breath. Bone marrow aspirates and biopsy

showed hypercellularity, being mainly erythroid progenitors, constituting >80% of bone marrow cell count with >30% proerythroblast without a significant myeloblastic component.

I examined that, majority of the patients had Macronormoblastic Erythroid Hyperplasia [48 (48.0%)] and it was statistically significant ($p < .00001$), ($z = 7.7273$)

I found that, significantly higher of patients had IDA [30 (30.0%)]. ($p < .00001$), ($z = 5.6662$)

Chabot-Richards DS et al [11](2017) found that numerous non-neoplastic and neoplastic conditions manifest with distinctive features in blood. The differential diagnostic considerations in blood smears with RBC destruction was presented, expanding beyond microangiopathic hemolytic anaemia. Lymphocyte morphologic assessment guides differential diagnosis including the identification of rare germline disorders.

Poorana Priya P et al [12] (2014) pancytopenia is a common hematological

entity encountered in their laboratory practice. ARC was calculated and an algorithm to approach the cases of pancytopenia was derived considering ARC as the key factor. A total of 429 cases of pancytopenia were evaluated and based on their algorithm results were tabulated. ARC was found to be $< 25 \times 10^9/L$ for cases of Aplastic anaemia; $25-50 \times 10^9/L$ for Nutritional anaemia; and, $> 100 \times 10^9/L$ in Marrow infiltrative disorders and in cases of sepsis and malaria. They found that ARC plays an important role in differentiating various causes of pancytopenia and hence should be routinely included in pancytopenia work up in order to avoid unnecessary bone marrow aspirations in pancytopenic patients. [13]

In my study, the mean Age of patients was 26.470 ± 16.0405 , the mean Hb (gm/dl) of patients was 8.6190 ± 1.7213 , the mean RBC (million/cumm) of patients was 2.8812 ± 0.8450 , the mean Ret % of patients was 1.4586 ± 0.9515 and the mean ARC ($10^9/L$) of patients was 43.4714 ± 33.6967 .

Table: Distribution of PBS

PBS	Frequency	Percent
Anemia & Thrombocytopenia	5	5.0%
Anemia, Thrombocytopenia, shift to left	2	2.0%
Dimorphic	4	4.0%
Falciparum Gametocyte	2	2.0%
Lymphocytosis	1	1.0%
Macrocytic Normochromic	18	18.0%
Microcytic Hypochromic	30	30.0%
Pancytopenia	9	9.0%
Pancytopenia & Lymphocytosis	8	8.0%
Ring form	3	3.0%
Schistocytes	9	9.0%
Target cells, Nucleated RBC	5	5.0%
Thrombocytosis	1	1.0%
vivax gametocyte	3	3.0%
Total	100	100.0%

Table: Distribution of BM Finding

BM Finding	Frequency	Percent
Erythroid Hyperplasia	11	11.0%
Increased Megakaryocyte	1	1.0%
LD Bodies & Increased Plasma cells	9	9.0%
Lymphoblast (>70%)	2	2.0%
Lymphoblast (>80%)	2	2.0%
Lymphoblast (> 40%)	2	2.0%
Lymphoblast (>60%)	1	1.0%
Lymphoblast (>30%)	2	2.0%
Macronormoblastic Erythroid Hyperplasia	48	48.0%
Myeloblast (> 30%)	3	3.0%
Myeloblast (> 60%)	2	2.0%
Myeloblast (>40%)	2	2.0%
Reactive Erythroid Hyperplasia	10	10.0%
Reversed M:E ratio	5	5.0%
Total	100	100.0%

Table: Distribution of Diagnosis

Diagnosis	Frequency	Percent
ALL	9	9.0%
AML	7	7.0%
Drug Induced hemolytic anemia	6	6.0%
Essential Thrombocythemia	1	1.0%
IDA	30	30.0%
IDA on Treatment	2	2.0%
MAHA	3	3.0%
Malaria	8	8.0%
Megaloblastic Anemia	18	18.0%
Megaloblastic Anemia on treatment	2	2.0%
Thalassemia	5	5.0%
Visceral Leishmaniasis	9	9.0%
Total	100	100.0%

Table: Distribution of mean Hb (gm/dl), RBC (million/cumm), Ret% and ARC($10^9/L$)

	Number	Mean	SD	Minimum	Maximum	Median
Hb(gm/dl)	100	8.6190	1.7213	3.4000	11.1000	9.1000
RBC (million/cumm)	100	2.8812	0.8450	0.8000	5.0100	2.9550
Ret%	100	1.4586	0.9515	0.1700	4.2300	1.1000
ARC ($10^9/L$)	100	43.4714	33.6967	2.0000	134.0000	23.7600

Conclusion

In my study, out of 100 patients most of the patients were 21 to 30 years old, which was statistically significant.

Present study showed that, higher number of the patients were male. Male: Female

ratio was = 1.8:1. Which was statistically significant.

I found that, most number of patients had Microcytic Hypochromic. It was statistically significant.

I examined that, majority of the patients had Macro-normoblastic Erythroid Hyperplasia and it was statistically significant.

I found that, significantly higher number of patients had IDA.

In my study, the mean Age of patients was 26.470 ± 16.0405 , the mean Hb (gm/dl) of patients was 8.6190 ± 1.7213 , the mean RBC (million/cumm) of patients was 2.8812 ± 0.8450 , the mean Ret % of patients was 1.4586 ± 0.9515 and the mean ARC ($10^9/L$) of patients was 43.4714 ± 33.6967 .

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