Original Research Article

ROLE OF RETICULOCYTE COUNT AND ITS INDICES IN EVALUATION OF PANCYTOPENIA CASES AT TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Pancytopenia means a reduction in the WBC, Hb and platelet count and is most often the result of anticancer chemotherapy, HIV infection, hypersplenism and bone marrow infiltration or failure. The reticulocytes are young Red Blood Cells (RBCs) that form a reticulum network or granules on exposure to those supravital stains. The reticulocyte count may be used as an index of red cell production in anemia and other hematological disease provided allowances are made for the production of in total red cell count.

Aims and Objective: To study and inerpret the role of reticulocytes and its indices for differential diagnosis of pancytopenia and to assess the bone marrow erythropoietic activity using reticulocyte and its indices.

Material and method-Prospective study of 150 cases of was carried out from June 2021 to November 2022 at Pathology department, Tertiary Care Hospital. Giemsa stain for peripheral blood smear and new methylene blue stain for reticulocyte count was done for manual method and Backman coulter for automated method for reticulocyte count was used.

Result: Megaloblastic anemia was more common in patients with vegetarian diet. Macroovalocytes on peripheral smear was a better predictor of Megaloblastic anemia. followed by Dual deficiency anemia (8.67%) and followed by Aplastic anemia (8%). Mean Absolute reticulocyte count(ARC) was 57.65. Lowest ARC 12.32 seen in Aplastic anemia and highest ARC 108.22 seen in Sepsis. Reticulocyte production index(RPI) of >2 seen in Hemolytic Anemia and Sepsis, while in other cause of pancytopenia RPI was <2 which shows decreased erythropoiesis.

Conclusion: All reticulocyte parameters are helpful to differentiate the various causes of pancytopenia, hence should be used in routine for early diagnosis and treatment in cases of pancytopenia, thereby avoiding unnecessary bone marrow aspiration or biopsy.

Key word: Reticulocytes counts and its indices, pancytopenia

INTRODUCTION

Pancytopenia means a reduction in the WBC, Hb and platelet count and is most often the result of anticancer chemotherapy, HIV infection, hypersplenism and bone marrow infiltration or failure.[1] It is not a disease entity but a triad of findings that may result from a number of disease processes — primarily or secondarily involving the bone marrow.[2] Although it is common hematological entity, there is relatively little discussion about it in many textbooks of Hematologic & Internal Medicine.[3]

Patient with pancytopenia presents with different clinical features. Marrow cellularity and composition in cases of pancytopenia differ in relationship to underlying pathological condition. The marrow is generally hypocellular in cases of pancytopenia caused by a primary production defect. Cytopenia resulting from ineffective hematopoiesis, increased peripheral utilization or destruction of cells, and bone marrow invasive processes are usually associated with a normocellular or hypercellular marrow. It has been observed in the Indian scenario that megaloblastosis (vitamin B12 and/or folate deficiency) is the commonest cause of pancytopenia.[4,5]

The reticulocytes are young Red Blood Cells (RBCs) that form a reticulum network or granules on exposure to those supravital stains. The reticulum network or granules represent precipitated rough endoplasmic reticulum with associated polyribosomes. Fully automated instruments provide a measure of the various degrees of reticulocyte maturation because the most immature reticulocytes, produced when erythropoietin levels are high, have more RNA and fluoresce more strongly than the mature reticulocytes normally present in the peripheral blood. An assessment of reticulocyte maturation can be important for diagnosing the cause of anemia and assessing the degree of effective erythropoiesis.[1]

The presence of increased polychromasia of the macrocytes on the peripheral smear and a reticulocyte count of >10% should raise suspicion of hemolysis or an acute bleed. These large polychromatophilic erythrocytes noted on the peripheral smear represent reticulocytes, immature RBCs that are larger than mature RBCs, and are indicative of increased erythropoiesis or RBC production and, if present in increased number, can raise the MCV. Additionally, the reticulocyte maturation parameters performed on the peripheral blood may also be helpful to differentiate megaloblastic from hemolytic causes of the macrocytosis.[6] Significant polychromasia is a reflection of an increased percentage of reticulocytes, but there is not necessarily an increased absolute reticulocyte. The polychromatic erythrocytes visible on a blood film corresponds to young reticulocytes with high amount of residual RNA. Polychromasia can be seen in conditions like,[7] Neonates, as a response to hemorrhage or hemolysis, in response to iron or B12/folate theraphy, during recovery from bone marrow failure, bone marrow infiltration Indices of reticulocyte by manual method are 1) Reticulocyte percent (%), 2) Absolute reticulocyte count (normal- 50000 to 85000/cumm), 3) Corrected reticulocyte count, (Corrected reticulocyte count > 2% indicates reticulocyte release appropriate for the degree of anemia. If <2%, reticulocyte release is inappropriate.) and 4) Reticulocyte maturation production index.

Normocytic and Normochromic Anemia has many causes. A useful approach is evaluation of the erythrokinetics in a given patient. Often, the reticulocyte production index (RPI) and examination of the bone marrow will suffice. The RPI is the simplest measure of effective erythropoiesis.

Beckman Coulter was among the first to offer reticulocyte enumeration technology on their STKS™, MAXM™, and MAXM A/L instruments. The Coulter technique utilizes a new methylene blue stain and differentiates reticulocytes from mature RBCs, white cells, and platelets through measurement of impedance, radiofrequency, and laser light scatter (VCS technology). The computer software provides a reticulocyte percentage, absolute reticulocyte count, mean reticulocyte volume, and maturation index in 60–120 sec based on examination of 32,000 cells.

MATERIAL AND METHOD-

This prospective study was carried out from June 2021 to November 2022 at Pathology department, Tertiary Care Hospital. The cases were selected on the basis of inclusion criteria after taking written & informed consent.

Sample size: 150 patients with pancytopenia.

Source of data: All the samples which came from various clinical wards like medical ward, surgery ward, TB & chest ward and orthopedic ward at Tertiary care Hospital.

Sample for study- Samples were then further preceded in the Laboratory of pathology department at Tertiary Care Hospital. Sample was taken in EDTA and Plain vaccute for complete blood count and biochemical or microbiological profile respectively. From Samples, First EDTA blood sample was run in automated hematology cell counter, and then a peripheral smear was made on glass slide and were stained in Giemsa stain and new methylene blue stain. Stained slides were examined microscopically.

RESULT

In this study, out of 150 cases, 88 cases (58.67%) were male while 62 cases (41.33%) were females. The male to female ratio was 1.42: 1. Thus there was a male preponderance. In present study, the maximum numbers of cases were noted in the age group of 21-30 years (38.67%) followed by 31-40 years (30.67%), while minimum numbers of cases were in age group of 61-70 years (2%). Hemoglobin percentage varied from 1.5 – 9.8 gm %. Most of patients had hemoglobin percentage between 5.1-7 gm % (moderate anemia). Lowest value of 1.5 gm% was seen in Aplastic anemia. Most of patients have total WBC count were between 2100-3000 cells/mm³. Most of patients were present with platelet count between 51000-100000 cells/cumm. In present study, non-malignant conditions were more commonly seen than malignant condition as a causative etiology of pancytopenia. In non-malignant condition, Nutritional anemia was more common in 64% cases. In Nutritional anemia, Megaloblastic

anemia was more common constituting the 55.33% of the cases followed by Dual deficiency anemia (8.67%). In present study, most of patient 52.67% had reticulocytes count was between 0.5-2.5% showing normal erythropoietic activity. In 11% patients, reticulocyte count was >5% showing increased erythropoietic activity. In 14.67% patients, reticulocyte count was <0.5% showing decreased erythropoietic activity. In present study, highest reticulocyte count seen in Hemolytic anemia which had mean manual reticulocyte count was 11.60±4.74 and mean automated reticulocyte count was 10.99±4.76(p=0.80). While lowest reticulocyte count was seen in Apalstic anemia which had mean manual reticulocyte count was 0.17±0.12 and mean automated count was 0.35±0.31(p=0.08). No statistically significant difference was found between the automated and manual count in all causes of pancytopenia. In present study, mean ARC is 51.45±21.34 x 10^9/L, mean CRC is 1.25±1.40%, mean MRV is 121.79±19.67 fl and mean IRF is 0.49±0.18.
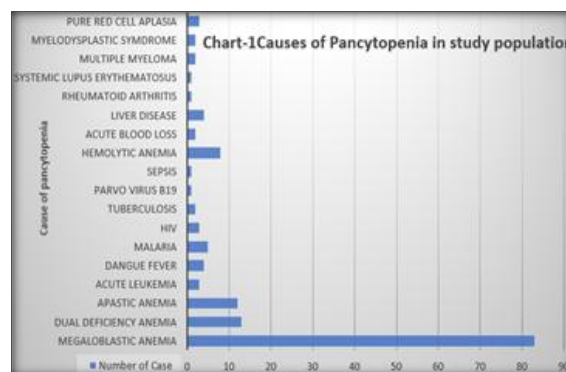

Chart 1

Table 1: Distribution of Number of cases according to Reticulocyte%

Reticulocyte%	No of cases	Percentage (%)
<0.5	22	14.67
0.5-2.5	79	52.67
2.6-5	38	25.33
>5	11	7.33

Table 2: Comparison of mean reticulocyte count between male and female by automated and manual method

Sex	Mean manual reticulocyte count	Mean automated reticulocyte count	p value
Male	2.71±3.26	2.37±3.11	0.48
Female	2.55±2.73	2.21±2.59	0.48

Chart 2

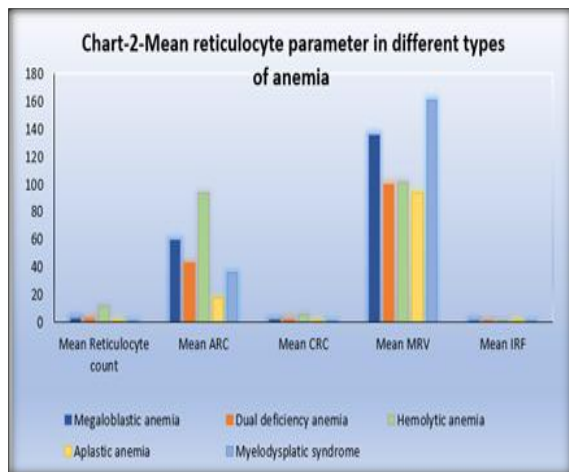**Figure 1: Peripheral smear shows hypersegmented neutrophil in Megaloblastic anemia (Giemsa stain 100x)**

In this study highest Mean Reticulocyte count, Mean ARC, Mean CRC and Mean IRF seen in hemolytic anemia. While highest Mean MRV seen in MDS. Aplastic anemia was presented with all lowest parameters.
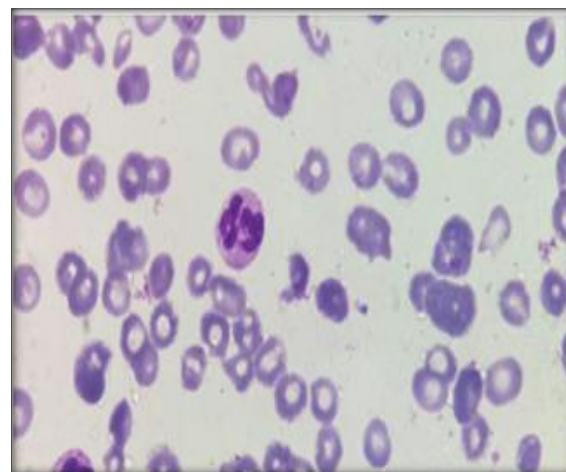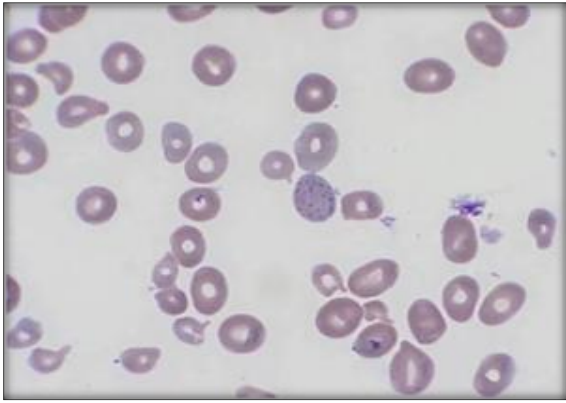
Figure 2: Peripheral smear showing basophilic stippling in case of Megaloblastic anemia (Giemsa stain 100x)
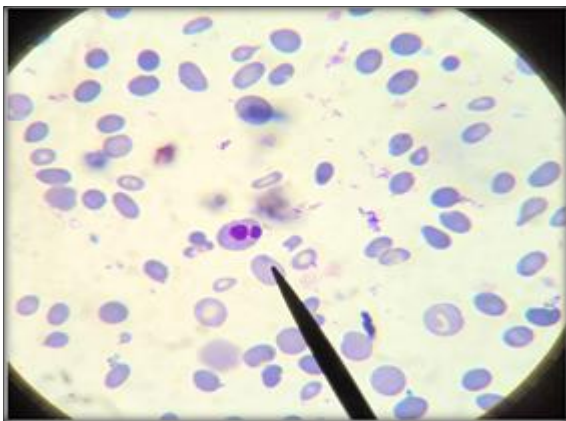

Figure 3: Peripheral smear of MDS – Erythroblast showing nuclear lobulation. (Giemsa stain 100x)
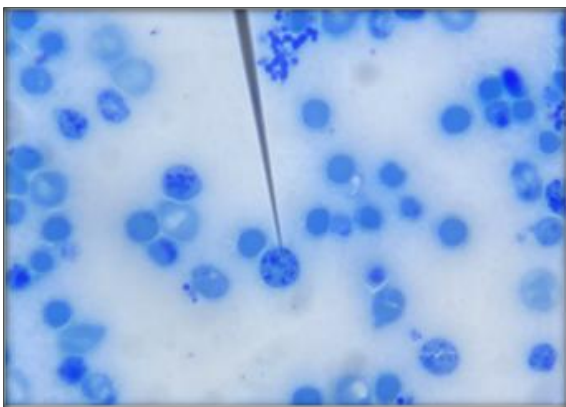

Figure 4: Reticulocyte stage III-Small reticulum along with scattered granules in hemolytic anemia (Mew methylene blue stain 100X)

DISCUSSION

In present study Male: Female ratio was 1.42:1. Male predominance was there as compared to female in present study, which was in accordance with Chhabra A. et al,[8] (1.64:1). This was in accordance with the other studies Rathod GB. et al,[9] (1.85:1) and Syed Nadeem et al,[10] (1.5:1) which found male predominance in pancytopenia cases. This male predominance could be due to social and cultural taboos in society, making health care facilities more readily available to males as compared to females which lead to increased male presentation at hospitals mainly in rural areas. The results of current study are comparable with other studies.

The commonest cause of pancytopenia, reported from various studies throughout the world has been Aplastic anemia. This is sharp contrast with the results of present study, where the commonest cause of pancytopenia was Megaloblastic anemia (55.33%). This seems to reflect the higher prevalence of nutritional deficiency of vitamin B12 and folic acid in Indian subjects. Similar results have been reported in other studies.[5,11]

The variations in the frequency of various diagnostic entities causing pancytopenia attributed to difference in methodology and stringency of diagnostic criteria, geographical area, study period of observation, genetic differences, prevalence of infection and varying exposure to myelotoxic agents, etc.

In present study, MRV was increased in megaloblastic anemia, MDS and decrease in aplastic and dual deficiency anemia. Other study like Gomez AT et al,[6] Gorte TR et al,[12] and Das J et al,[13] also shows increased MRV in megaloblastic anemia.

In present study, highest MRV (162 fl) seen in MDS. Other study like Gomez AT et al,[6] Gorte TR et al,[12] and Das J et al,[13] also shows highest MRV in MDS. Cut of value of MRV of >121 fl which differentiate macrocytic anemia (Magaloblastic anemia, Liver disease and MDS) from other cause of pancytopenia. The MRV value further classifying MDS had cut off of >159 fl which differentiated from Liver disease and Megaloblastic anemia. It can further re classified Megaloblastic anemia which has cut off of >127 fl which differentiated it from MDS. Same findings seen in study of Das J et al.[13]

In present study, IRF of Megaloblastic anemia and Dual deficiency anemia is same as Das J et al,[13] study. Gomez AT et al,[6] also reported that IRF of Megaloblastic anemia (0.38) higher than normal. Present study also shows similar findings.

IRF is early and sensitive parameter for erythropoiesis. Higher the IRF, greater the erythropoietic activity. IRF is very useful parameter to evaluating erythropoietic activity and clinical utility is greatest in classification of anemia based on marrow response.

The cut off value of IRF of > 0.80 seen in hemolytic anemia, acute blood loss, hypersplenism which can be differentiated from neoplastic condition like acute leukemia, multiple myeloma which had IRF <0.80. The case with IRF < 0.58 were further classified Megaloblastic anemia and Dual deficiency anemia shows value between 0.38 -0.58 which can be differentiated from MDS, Liver disease, SLE, Rheumatoid arthritis, Aplastic anemia and Pure red cell aplasia which had IRF value of <0.38. Cases with <0.38 IRF further classified with cut of value of 0.19. It helpful to differentiation of MDS, Liver disease, SLE, Rheumatoid arthritis which had IRF value of >0.19 from Aplastic anemia and Pure red cell aplasia

which had value of <0.19. Das J et al,[13] study also had same findings.

CONCLUSION

The present study showed that the commonest cause of pancytopenia was Megaloblastic anaemia. Reticulocyte parameter and peripheral smear examination had an important role in the differential diagnosis and management of pancytopenia.

The automated method is preferred as it is fast, highly precise and useful in conditions where reticulocyte parameters are required such as IRF and MRV.

There was difference in ARC values in various causes of pancytopenia, so it might be helpful in preliminary diagnosis of pancytopenia based on ARC.

MRV is most useful parameter to differentiating the causes of macrocytic anemia for further evaluation of pancytopenia.

RPI is also useful parameter of erythropoiesis to evaluate ether marrow is active or not. But this parameter is replaced by IRF which is early parameter to rise, in case of increase marrow erythropoiesis before increment of other reticulocyte parameters, hence, it can be helpful for early diagnosis.

IRF is an early parameter useful for diagnosis, which gives an idea of bone marrow recovery. The clinical utility of IRF is useful for diagnosing type of anemia and its treatment.

All reticulocyte parameters are helpful to differentiate the various causes of pancytopenia, hence should be used in routine for early diagnosis and treatment in cases of pancytopenia, thereby avoiding unnecessary bone marrow aspiration or biopsy.

Limitation of Study

In this study limitation include lack of comparison groups, difficulty in generalizing findings to large population. There may lack a control group or comparison group of patient with other hematological conditions, making it difficult to isolate the specific impact of reticulocyte parameter in pancytopenia. In severe anemia, reticulocyte may have longer lifespan, which can lead to falsely elevated corrected reticulocytes count.

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