

Original Research Article

Evaluation of red cell indices and manual reticulocyte parameters in iron deficiency, vitamin B12 deficiency and beta thalassemia minor patients in a tertiary care hospital

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ABSTRACT

Background: Reticulocytes are the youngest erythrocytes released from the bone marrow. Their increase or decrease can be an indicator of erythropoietic activity or failure. Manual reticulocyte count is an assay traditionally used to evaluate the status of erythropoiesis. Reticulocyte parameters could be cost effective methods to guide the clinician in treating anaemias. Aim of current study was to evaluate the role of routine red blood cell parameters and reticulocyte parameters in differentiating various anaemias.

Methods: This retrospective cross-sectional study was conducted by retrieving the laboratory parameters of patients admitted at Yenepoya medical college hospital from January 2021 to July 2021, duration of 6 months. Fifty cases of anaemia were included. These included records of patients with anaemia based on Serum Ferritin levels, Vitamin B12 levels, and beta thalassemia cases confirmed using electrophoresis. Other various parameters like mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were also taken into consideration for descriptive statistics.

Results: Statistically significant correlation was observed for reticulocyte count, reticulocyte production index and other red blood cell parameters like MCV, MCH and MCHC between iron and vitamin B12 deficiency anaemias. No statistically significant correlation was observed in the beta thalassemia group but specifically showed significance in MCV.

Conclusions: Reticulocyte count and reticulocyte production index and good markers apart from the routinely used traditional red blood cell parameters in differentiating between iron deficiency and vitamin B12 deficiency anaemias. However, in beta thalassemia minor cases, the mean corpuscular volume is a better marker. Present study showed male gender predominance in anemia which could be due to ethnic and topographical variations.

Key words: Anaemia, Haemoglobin, Haematocrit, Red cell indices, Reticulocyte count, Reticulocyte production

INTRODUCTION

Reticulocytes are the youngest erythrocytes released from the bone marrow into the blood; generally circulate for 1-2 days before becoming mature erythrocytes.¹ Any increase or decrease in their count could serve as an indicator of erythropoietic activity or failure, such as in

anaemias or bone marrow dysfunction. Reticulocyte count estimation via manual method has been an assay traditionally used for the purpose of evaluating the status of erythropoiesis in haematological disorders with disturbances in erythropoietic activity, especially of use in anaemias.² The traditional erythropoietic parameters like mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) have been routinely used in the evaluation of anaemia.^{1,2} In an anaemic individual, the bone marrow is generally unable to respond, resulting in a low reticulocyte count. In contrast, when the bone marrow can respond appropriately, an increase in reticulocyte count can be observed. A reduction in reticulocyte count can be caused by various conditions like hypochromic anaemia, aplastic anaemia, nutrition deficiency anaemia, aplastic crisis in haemolytic anaemia and myelodysplastic syndromes². Reticulocyte count in a healthy person is usually in the range of 0.5% to 2.5%.³ The characteristic haematological finding of iron deficiency anaemia (IDA) is decreased MCV, microcytosis in the peripheral blood smear and hypochromia in the erythrocytes.^{3,4}

$$\text{Corrected Reticulocyte Count (CRC)} = \frac{[\% \text{ reticulocyte count} \times \text{Patient's Haematocrit}]}{45 \text{ (normal Haematocrit)}}$$

$$\text{Reticulocyte production index (RPI)} = \frac{\text{Corrected reticulocyte count}}{\text{Reticulocyte maturation time}}$$

Reticulocyte production index (RPI), which is derived from corrected reticulocyte count is also used in evaluation of anaemias. At times, a falsely elevated reticulocyte count can be observed when a person is anaemic. A low RBC count can in turn increase the reticulocyte count even if erythropoiesis has not taken place because the reticulocyte count represents a percentage in comparison to the RBC count. There exists a high possibility that an appropriate increase in erythropoiesis has not taken place in response to anaemia, hence the corrected reticulocyte count (CRC) remains an applicable method.⁴ In addition to routine red cell parameters, manual reticulocyte count and RPI are cost effective methods to guide the clinician to identify various types of anaemias and also helps in the therapeutic management and follow up, as it is an indirect method to assess the bone marrow function.

METHODS

This was a retrospective cross-sectional study. This study was conducted by retrieving records (laboratory parameters) the admitted patients from the archives of the central laboratory at a tertiary care hospital. The study was conducted from January 2021 to July 2021, duration of 6 months. A total of 50 cases of anaemia were documented, and included records of patients with anaemia based on Serum Ferritin levels, Vitamin B12 levels and beta thalassemia cases confirmed using electrophoresis. Apart from the manual reticulocyte count and calculated RPI, various other routine red blood cell parameters like MCV, MCH and MCHC were also recorded. Hemolytic anaemia and anaemia of chronic disease were not included in the study. Ethylene diamine tetra acetic acid (EDTA) anticoagulated blood sample of

in-patients and out-patients who visited the hospital during the course of the study were collected and run in the Sysmex 6 parts haematology automatic cell counter-Sysmex XN-1000 automator. Other routine parameters like haemoglobin, haematocrit, RBC indices (MCV, MCH and MCHC) were also recorded. The slides for reticulocyte count estimation were prepared by mixing the blood sample with equal amounts of new methylene blue dye. The mixture was then incubated at 37°C for 15 minutes, followed by preparation of the slide. After drying the slide, reticulocyte count estimation was done under oil immersion. The values of haemoglobin, haematocrit, other routine RBC indices and reticulocyte count parameters like reticulocyte count and RPI were recorded and documented in MS Excel 2019, SPSS (Statistical Package for Social Sciences) version 23 was used for statistical analysis.

RESULTS

The age and gender of our study population were recorded. Age of persons included in the study ranged from 7 months to 70 years with mean age of 40.4±17.6. In our study, anaemia was predominantly seen in seen in males (52%) compared to females (48%) (Figure 1).

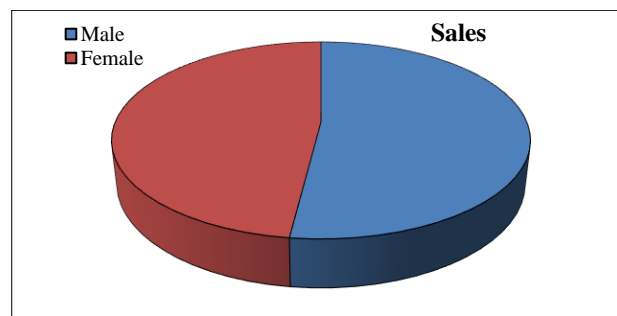


Figure 1: Gender wise distribution of anaemia.

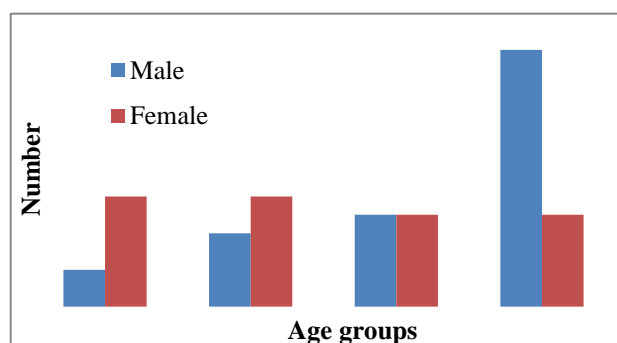


Figure 2: Age and gender wise distribution of anaemia.

Anaemia was most commonly seen in the age group of 31 to 70 years (Figure 2). Using Chi Square test, no statistically significant difference was observed in haematocrit level on the basis of age groups ($p > 0.05$) (Figure 3). Comparison of mean MCV values across

different age groups is shown in Figure 4. This shows that all iron deficiency anaemia and beta thalassemia minor patients had low MCV whereas Vitamin B12 deficiency anaemia has high MCV values. Decreased MCV and MCH are suggestive of microcytic hypochromic anaemia.

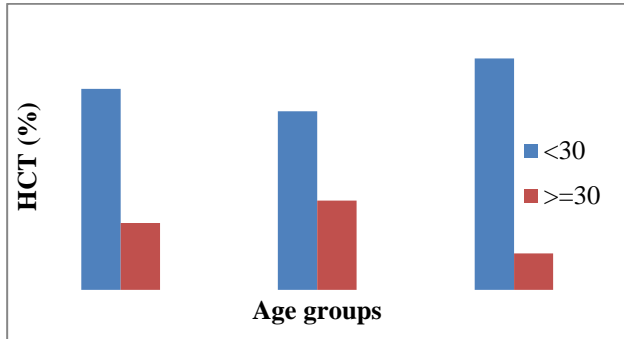


Figure 3: Age wise comparison of haematocrit levels.

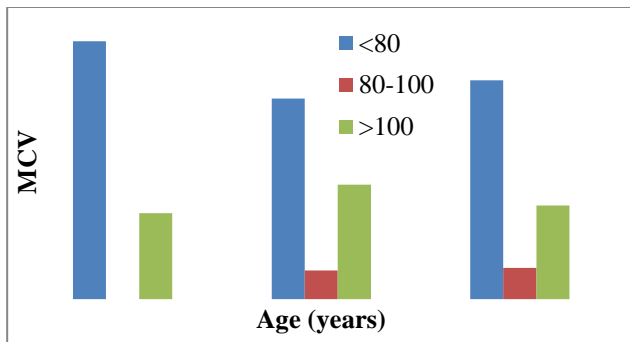


Figure 4: Comparison of MCV values in different age groups.

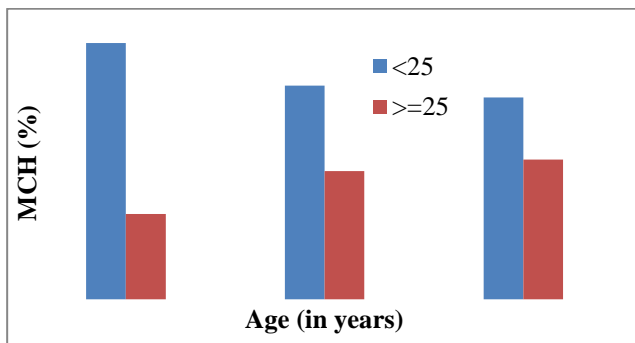


Figure 5: Age wise comparison of MCH values.

Using Chi Square test, no statistically significant difference was observed in MCV and MCH on the basis of age groups ($p > 0.05$) (Figure 4-5). MCHC decreases when MCV is reduced as it is a measure of the average concentration of haemoglobin inside a single RBC. The lowest value of MCHC was seen in anaemic participants who showed low MCV values (Figure 6). Reticulocyte count was decreased in both groups of anaemia with the decrease being more in the iron deficiency anaemia group (Figure 7). Diagonal segments are produced by ties. In

addition to the routine laboratory parameters like MCV, MCH and MCHC, the calculated reticulocyte production index (RPI) can also be used to differentiate Iron deficiency anaemia from Vitamin B12 deficiency anaemia (Figure 8). ROC curve analysis showed statistically significant values in the parameters- MCV, MCH, MCHC and reticulocyte production index in the two groups namely the Vitamin B12 deficiency and Iron deficiency anaemia groups. There were no statistically significant values in beta thalassemia minor groups.

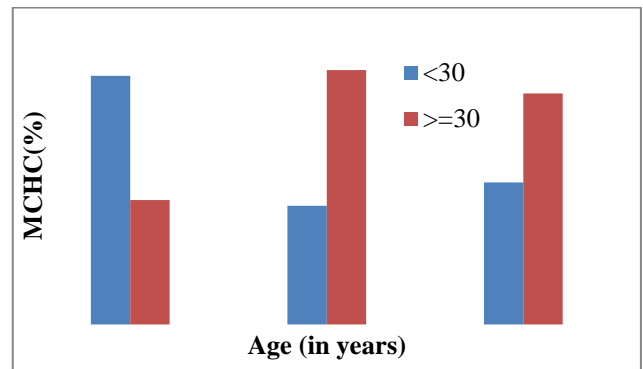


Figure 6: Age wise comparison of MCHC values.

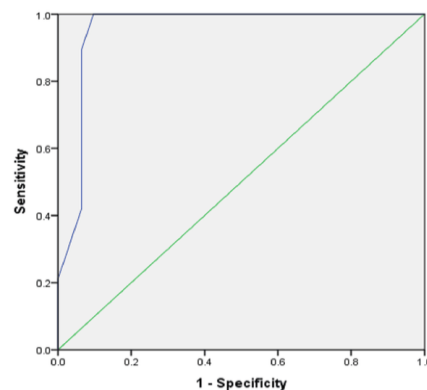


Figure 7: ROC curve analysis of reticulocyte count in iron deficiency and vitamin B12 deficiency anaemia groups.

DISCUSSION

Anaemia affects both developing and developed countries and is seen affecting a large population worldwide^{5, 6}. Prevalence of anaemia across different part of India is different ranging from 8.7% to 53.2%.⁷⁻⁹ The most prevalent type of anaemia being nutritional anaemia is generally due to a lack of iron, folate, or vitamin B12.¹⁰ Iron deficiency anaemia is usually straight forward to diagnose, but due to its generic clinical indications, it often remains untreated for a long period.¹¹ In our study, mean age of the population with anaemia was 40.4 ± 17.6 with the age ranging from as young as 7 months to a maximum of 70 years. Majority belonged to the age group ≥ 15 years (84%). A study done in different parts of India showed similar findings with majority of anaemic

cases belonging to the adult age group.^{9,12,13} Anaemia was noticed to be more common in males (52%), mildly elevated compared to females (48%) in the present study.

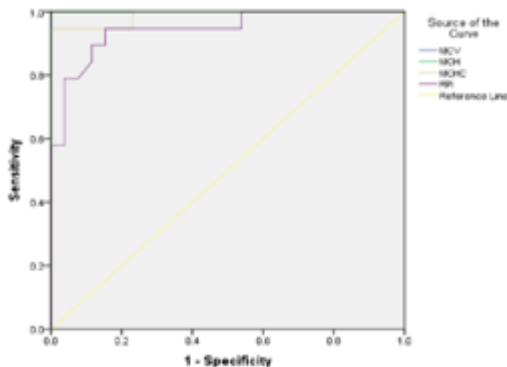


Figure 8: ROC curve analysis of reticulocyte production index, MCV, MCH and MCHC in the iron deficiency and vitamin B12 deficiency anaemia groups.

However, the findings in the present study were not in agreement with majority of studies performed in India where a greater number of females were found to be anaemic. The results of the study conducted by Pradeep Kumar et al, regarding the burden of anaemia among men in the 'empowered action group states' showed that, the prevalence of anaemia among men was reported to be higher in comparison to the national average. A similar pattern of prevalence of anaemia was seen among men across all empowered action group states.¹⁴ In the ROC curve analysis, MCV and MCHC were found to be the more significant parameters with 96% sensitivity and 100% specificity followed by reticulocyte count and RPI with 87% sensitivity and 100% specificity. Reticulocyte count and RPI were statistically significant in our study to differentiate between vitamin B12 deficiency and iron deficiency anaemia. The reticulocyte count apart from giving valuable insight into various aspects of erythropoietic control, it also reflects the balance of production of red cells in relation to their rate of destruction.¹⁵ No significant parameter was defined between iron deficiency and beta thalassemia minor patients within these reticulocyte parameters in ROC analyses. The classification of anaemia is based on the alteration in the red cell morphology and it also points to a particular cause. Morphological characteristics like normocytic, microcytic or macrocytic are known to provide etiological clues. In the study, low MCV was seen in iron deficiency anaemia group and high MCV was seen vitamin B12 deficiency anaemia group. This showed microcytic picture predominantly in children and women, macrocytic picture in cases aged ≥ 15 years. MCV was found to be more significant in the thalassemia group. Hence, microcytic anaemia was found to be the predominant type of anaemia in the present study. These findings are in consensus with the studies done by Jadhav et al and Qureshi et al and Kerdany et al in which

microcytic anaemia cases were the predominant type.^{5,7,15} Several studies revealed that microcytic hypochromic anaemia was common and iron deficiency was found to be the commonest aetiology.^{8,12,13,16-20} Macrocytic anaemia was quite common among older adults and comprised of almost 14% of all anaemia cases. Macrocytosis is most commonly associated with vitamin B12, folic acid deficiency followed by alcoholism and liver disease. In our study low MCH and low MCHC was very common in iron deficiency anaemia group. MCH is usually decreased in hypochromic anaemia. This finding was in consensus with other studies that showed that MCH and MCHC was reduced in most of the anaemic cases.^{5,13,20} Sex wise comparison of variables like Haematocrit, MCV, MCHC were not statistically significant. Prevalence of anaemia increases with age and among different age groups. Haematological parameters in various types of anaemia are different among various age groups. It gives us an idea about the usage of the morphological classification of anaemia, to aid in diagnosing various causes of anaemia. Microcytic hypochromic anaemia was found to be common in children and in adults, macrocytic anaemia was found to be more prevalent. Routine haematological parameters are very simple tests which can be easily obtained from automated haematology cell counters. Manual reticulocyte count and RPI are cost effective measures which can aid in diagnosis of different types of anaemias. They are of utmost importance in classifying anaemias and for further patient management.

Limitations

The study is limited due to small sample size and topographic and ethnic variation. Hence the study results cannot be generalised.

CONCLUSION

Reticulocyte count and reticulocyte production index and good markers apart from the routinely used traditional red blood cell parameters in differentiating between iron deficiency and vitamin B12 deficiency anaemias. However, in beta thalassemia minor case the mean corpuscular volume is a better marker. These are cost effective methods which can aid in diagnosis of various anaemias. Current study showed male gender predominance in anemia which could be due to ethnic and topographical variations.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Ceylan C, Miskioğlu M, Colak H, Kiliccioğlu B, Özdemir E. Evaluation of reticulocyte parameters in iron deficiency, vitamin B(12) deficiency and beta-

- thalassemia minor patients. *Int J Lab Hematol.* 2007; 29:327-34.
2. Watanabe K, Kawai Y, Takeuchi K, Shimizu N, Iri H, Ikeda Y, et al. Reticulocyte maturity as an indicator for estimating qualitative abnormality of erythropoiesis. *Am J Clin Pathol.* 1994;47:736-9.
 3. Raja-Sabudin RZ, Othman A, Ahmed-Mohamed KA, et al.: Immature reticulocyte fraction is an early predictor of bone marrow recovery post chemotherapy in patients with acute leukemia. *Saudi Med J.* 2014;35:346-9.
 4. Piva E, Brugnara C, Spolaore F, Plebani M. Clinical utility of reticulocyte parameters. *Clin Lab Med.* 2015;35:133-63.
 5. Jadhav SU, Khaparde S: Study of the red cell indices, hemogram and platelet variations in anemic (10 gm %) patients by automatic cell counter in a tertiary care centre, Ahmednagar, Maharashtra, India. *Int J Res Sci.* 2017;5:1582-8.
 6. Pudasaini S, Pant PP, Kafle N, Maharjan S, KCA, Shrestha S. Study of red cell indices and reticulocyte count in person with anemia at a tertiary care hospital of Kathmandu. *Nepal Med Coll J.* 2019;31:1-6.
 7. Qureshi NA, Chauhan MAZ, Goswami AP, Suri SK. Study of anemia and its correlation with hematological parameters in patient of various age group. *J Dental Med Sci.* 2015;14:29-35.
 8. Verma P, Singh S, Ghildiyal A, Kumar A, Krishna A: Prevalence of anemia in adults with respect to sociodemographic status, blood groups and religion in north Indian population. *Int J Biol Med Res.* 2012; 3:2422-8.
 9. Gupta SK, Agarwal SS, Kaushal R, Jain A, Gupta VK, Khare N. Prevalence of anemia among rural population living in and around of rural health and training center, Ratua village of Madhya Pradesh. *Muller J Med Sci Res.* 2014;5:15-8.
 10. Verma K, Baniya GC. Prevalence, knowledge, and related factor of anemia among school-going adolescent girls in a remote area of western Rajasthan. *J Family Med Primary Care.* 2022;4:1474-81.
 11. Bellizzi S, Pichierri G, Napodano CM, Salaris P, Fiamma M, Fozza C. Iron deficiency anaemia and low BMI among adolescent girls in India: The transition from 2005 to 2015. *Public. Health Nutr.* 2021;24:1577-82.
 12. Uria GA, Naik PK, Midde M, Yalla PS, Pakam R. Prevalence and severity of anemia stratified by age and gender in rural India. *Hindawi.* 2014;10:1-5.
 13. Jadhav MV, Agarwal SA, Kadgi NV, Khedkar SS, Kulkarni KK, Kudrimoti JK. Utility of automated RBC parameters in evaluation of anemia. *Intl J Healthcare Biomed Res.* 2015;3:170-81.
 14. Kumar P, Sharma H, Patel KK: Prevalence and risk factors of anaemia among men: a study based on empowered action group states, India. *Nutr Health.* 2021;27:191-8.
 15. Cortellazzi LC, Teixeira SM, Borba R, Gervasio S, Cintra CS, Grotto HZW. Reticulocyte parameters in hemoglobinopathies and iron deficiency anemia. *Rev Bras Hematol Hemoter.* 2003;25:97-102.
 16. Ali El KT, El Ela SA, Aziz JBS. Study of automated red blood cells parameters in correlation with routine RBCs morphology by smear review. *Egyp J Hosp Med.* 2017;68:1358-63.
 17. Baral KP, Onta SR: Prevalence of anemia amongst adolescents in Nepal: a community based study in rural and urban areas of Morang district. *Nep Med Coll J.* 2009;11:179-82.
 18. Niazii M, Tahir M, Raziq F, Hameed A. Usefulness of red cell indices in differentiating microcytic hypochromic anemias. *Gomal J Med Sci.* 2010;8:125-8.
 19. Patel KV. Epidemiology of anemia in older adults. *Semin Hematol.* 2008;45:210-7.
 20. Rairikar SS, Ahire AP, Baheti NR, Patil MA: A study of blood cell indices and reticulocytes count in cases of anemia in females in reproductive age group. *MVP J Med Sci.* 2014;1:56-60.

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