[Downloaded free from http://www.ijhonline.org on Wednesday, December 16, 2020, IP: 109.224.55.20]

Original Article

Access this article online

Quick Response Code:

Website: www.ijhonline.org

DOI: 10.4103/ijh.ijh_42_20

Automated versus manual method for reticulocyte count: A comparative study in rural central India

Trupti Ramkrushna Gorte, Abhay Vilas Deshmukh, Nitin M Gangane

Abstract:

BACKGROUND: Reticulocytes are immature red blood cells that contain remnants of ribonucleic acid. Various reticulocyte parameters can help in the proper diagnosis of different anemias. It can be measured by manual as well as by automated method.

OBJECTIVE: The aim is to compare between the manual and automated methods of reticulocyte count (RC) in anemia.

MATERIALS AND METHODS: It was a laboratory-based cross-sectional study in which the comparison of RC by manual and automated method along with various reticulocyte parameters was done in 300 patients with anemia and 300 control samples matched for age and sex.

RESULTS: The study cases included 146 females and 154 males. No statistically significant difference was found between the automated and manual count among the male ($P = 0.77$) as well as female patients ($P = 0.61$). No statistically significant difference was found in mean RC among the infants ($P = 0.71$), children ($P = 0.59$), and adults ($P = 0.66$) between automated and manual count. The difference between mean manual and automated RC was statistically significant only in the case of males in the macrocytic anemia group ($P = 0.092$). Among reticulocyte indices, mean immature reticulocyte fraction (IRF), mean reticulocyte volume (MRV), and reticulocyte hemoglobin cellular content (RHCC) was found to be statistically significant among all types of anemia ($P = 0.001$, 00001 and 0.0001 respectively) while it was insignificant in case of mean corrected RC ($P = 0.89$). A significant positive correlation was found between manual and automated RC method by using Pearson's correlation coefficient ($r = 0.985$, $P = 0.0001$).

CONCLUSION: There was no significant difference between the automated and manual methods for reticulocyte counting. However, the manual method may be preferred as it is cost-effective; yet, it is laborious, time-consuming, need efficient technique, not suitable for heavily loaded laboratories and may be suitable for under-resourced laboratories. However, the automated method is preferred as it is fast, highly precise, and it is mandatory for certain diseases where reticulocyte parameters are required as a statistically significant difference was found among the different parameters such as IRF, MRV, and RHCC.

Keywords:

Anemia, automated reticulocyte count, manual reticulocyte count, pentra XLR, reticulocyte parameters

Department of Pathology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, Maharashtra, India

Address for correspondence:
Dr. Nitin M Gangane, Department of Pathology, Mahatma Gandhi Institute of Medical Sciences, Sevagram - 442 102, Wardha, Maharashtra, India.
E-mail: ngangane@mgims.ac.in

Submission: 22-08-2020
Accepted: 16-09-2020
Published: 10-11-2020

Introduction

Careful assessment of the blood is the first step in the assessment of hematological function and diagnosis of the related diseases.[1] Reticulocytes are young or immature red blood cells that are released from bone marrow and that contain remnants of ribonucleic acid (RNA) and ribosomes.[2] Reticulocyte count (RC) is the index of erythropoietic activity within the bone marrow and its measurement provides an initial assessment of anemia.[1,2]

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Gorte TR, Deshmukh AV, Gangane NM. Automated versus manual method for reticulocyte count: A comparative study in rural central India. Iraqi J Hematol 2020;9:145-9.

[Downloaded free from http://www.ijhonline.org on Wednesday, December 16, 2020, IP: 109.224.55.20]

It is having great diagnostic and prognostic value in hemolytic anemias, in acute hemorrhage, to study the response to iron, folic acid and Vitamin B12 therapy as well as after chemotherapy.[3]

The RC can be done by either by manual or by an automated method. Manual RC by microscopy has been considered as the standard method since 1940 because of its simplicity and low-cost.[4] However, it presents some inconvenience and limitations such as lack of accuracy, more time required for analysis, lack of quality of the stain, and inappropriate blood films.[5] Automated RC, through continued instrument, software, and reagent developments, provides an improved precision for the RC. It is a rapid and simple investigation which assists in providing a sensitive approach to the diagnosis and therapeutic monitoring of the anemic patients.[6]

Automated reticulocyte counting makes the determination fast, specific, and efficient by counting a large number of red blood cells.[7] It can provide information about individual cell characteristics, such as hemoglobin content of reticulocytes, hemoglobin content of mature erythrocytes, percentages of microcytic erythrocytes and hypochromic cells, mRNA content and of cellular indices such as volume, hemoglobin concentration, and content. All these novel parameters are useful in reporting and interpretation in the diagnosis of specific anemia.[7]

There are very few reports in the literature regarding this aspect, especially in Central India. Hence, the present study was carried out to compare between the automated and manual method of RC in different anemias.

Materials and Methods

It was a laboratory-based cross-sectional study conducted in the Department of Pathology at Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, a rural tertiary care hospital in Central India over a period of 24 months (October 2016 to September 2018) after clearance from Ethics committee of the Institute. Both the cases and control samples were obtained from the Haematology section, from patients who were already being investigated for their illnesses. In this study, comparison of RC by the manual and automated method was done in 300 patients with anemia and 300 control samples which were matched for age and sex. The cases were selected randomly by systematic randomization (every alternate sample) with hemoglobin (Hb) <11 g/dL and control samples were collected from the same laboratory with Hb >11 g/dL. All the samples (both cases and controls) were processed for RC by manual as well as automated method within 4 h of sample collection.[8,9] We divided our cases into three different age groups according to age, which included infants (up to 1 year), children (>1 year to 14 years) and adults (>14 years) for comparison of mean RC. We also classified 300 patients of anaemia according to morphological types of anemia; mean corpuscular volume (MCV) <80 fL as microcytic, MCV = 80–100 fL as normocytic and MCV >80 fL as macrocytic.[9] The cases with microcytic anemia were 177, normocytic were 93, and macrocytic were 30 in numbers.

Method

For manual RC,[8] fresh ethylene diamine tetra-acetate (EDTA) anticoagulated blood samples were used. For each sample, aliquots of 50 µL of EDTA anticoagulated blood and 50 µL of Azure B stain were added to the polystyrene tube. The mixture was incubated for 30 min at 37°C protected from light. After remixing, blood films were prepared on clean glass slides for microscopy. The slides were allowed to dry for 10 min. Counting was performed using a microscope with ×100 objective lens in oil immersion field. The number of reticulocytes was calculated by counting the number of these cells in 1000 erythrocytes evaluated on each smear of all samples. The counting was performed by the two pathologists for control samples. The results were expressed as a percentage of cells containing stained RNA (reticulocyte).

For automated RC,[2] *Horiba Pentra XLR* was used. It was a fully automated hematology analyzer used for the *in vitro* diagnostic testing of whole blood specimens. Horiba Pentra XLR gives the various parameters like complete blood count along with differential counts and different reticulocyte parameters. The differential reticulocyte parameters include immature reticulocyte fraction (IRF), corrected RC (CRC), mean reticulocyte volume (MRV) and reticulocyte haemoglobin cellular content (RHCC).

Ethics

The study protocol was approved by the Institute Ethics committee of Mahatma Gandhi Institute of Medical Sciences, Sevagram, letter number MGIMS/IEC/PATH/100/2016, dated October 5, 2016. Patient confidentiality was maintained during all research procedures.

Statistical analysis

Statistical analysis was done by using descriptive and inferential statistics using *Z*-test for the difference between two means and Cronbach's alpha, *Z*-test, *F* test, and Pearson's correlation coefficient test. Software used was SPSS17.0 (IBM Corp. Released 2011. IBM Statistics for Windows, Version 20.0: Armonk, New York, United States) and graph pad PRISM 5.0 version

[Downloaded free from http://www.ijhonline.org on Wednesday, December 16, 2020, IP: 109.224.55.20]

(GraphPad PRISM, Version 5.0: San Diego, California, USA) and $P < 0.05$ was considered as the minimum level of significance.

Results

Basic demography in study cases showed 146 females and 154 males. Out of 300 cases, 22 cases were infants (up to 1 year), 62 cases were children (>1 year to 14 years) and 216 cases were adults (>14 years).

The mean automated RC for males was 4.71 ± 4.18 and it was 4.85 ± 4.34 by the manual method. In the case of females, the mean automated RC was 3.62 ± 3.44 and that by the manual method, it was 3.82 ± 3.66. No statistically significant difference was found between the automated and manual count among the male ($P = 0.77$) as well as female patients ($P = 0.61$) by z-test for the difference between the mean automated RC and mean manual RC [Table 1 upper half].

The mean automated RC among infants (up to 1 year) was 2.31 ± 1.47 and it was 2.13 ± 1.72 by manual method. In the case of children (>1 year-14 years), the mean automated RC was 3.89 ± 2.89 and 4.17 ± 3.02 by manual method. For adults (>14 years), the mean automated RC was 4.45 ± 4.22 and 4.63 ± 4.40 by manual method. *Z*-test for difference between two means was applied. No statistically significant difference was found in mean RC among the infants ($P = 0.71$), children ($P = 0.59$), and adults ($P = 0.66$) between automated and manual count [Table 1 lower half].

When we divided cases as per morphologic classification of anemia in both sex, the difference between mean manual and automated RC was statistically significant only in the case of males in the macrocytic anemia group ($P = 0.092$) while it was insignificant in rest cases and sex groups in other groups [Table 2].

When compared for reticulocyte indices, mean IRF, Mean MRV, and mean RHCC was found to be statistically significant among all types of anemia ($P = 0.001$, 00001, and 0.0001, respectively) while it was insignificant in the case of mean CRC ($P = 0.89$) [Table 3].

A significant positive correlation was found between manual and automated RC method by using Pearson's correlation coefficient ($r = 0.985$, $P = 0.0001$) [Table 4 and Figure 1].

Discussion

Our study showed no statistically significant difference between the mean automated RC and mean manual RC amongst males as well as females ($P = 0.77$ and $P = 0.61$). The literature shows studies with varied results which compared RC between males and females,[10,11] but not the methods. Thus, both methods are suitable for the determination of mean RC, but the manual method can be more preferred as it is cost-effective. We also did not find any significant difference among different age groups, i.e., infants, children, and adults ($P = 0.71$, 0.59, and 0.66) [Table 1]. The comparison of mean RC value by both manual and automated method

Table 1: Comparison of mean reticulocyte count between male and female by automated and manual method and comparison according to age

Sex (*n*=300)	Mean automated reticulocyte count (±SD)	Mean manual reticulocyte count (±SD)	*Z*-test (*P*)
Male (*n* =154)	4.71±4.18	4.85±4.34	0.29 (0.77)
Female (*n*=146)	3.62±3.44	3.82±3.66	0.49 (0.61)
Comparison of mean RC according to age			
Infants (upto 1 year) (*n*=22)	2.31±1.47	2.13±1.72	0.37 (0.71)
Children (>1–14 years) (*n*=62)	3.89±2.89	4.17±3.02	0.52 (0.59)
Adults (>14 years) (*n*=216)	4.45±4.22	4.63±4.40	0.43 (0.66)

SD=Standard deviation, RC=Reticulocyte count

Table 2: Mean reticulocyte count according to morphologic classification of anaemia in study cases

Anemia (*n*=300)	Automated reticulocyte count (±SD)	Manual reticulocyte count (±SD)	*Z*-test (*P*)
Microcytic anaemia (MCV <80) (*n*=177)			
Male (*n*=92)	4.63±4.20	4.74±4.44	0.1 (0.86)
Female (*n*=85)	3.26±2.65	3.62±2.80	0.84 (0.39)
Normocytic anaemia (MCV 80–100) (*n*=93)			
Male (*n*=46)	4.47±3.63	4.66±3.71	0.25 (0.80)
Female (*n*=47)	3.74±3.23	3.71±3.43	0.03 (0.97)
Macrocytic anaemia (MCV >100) (*n*=30)			
Male (*n*=16)	5.86±5.45	60.5±5.42	0.09 (0.092)
Female (*n*=14)	5.40±6.75	5.50±7.32	0.02 (0.97)

SD=Standard deviation, MCV=Mean corpuscular volume

[Downloaded free from http://www.ijhonline.org on Wednesday, December 16, 2020, IP: 109.224.55.20]

Table 3: Comparison between mean immature reticulocyte fraction, corrected reticulocyte count, mean reticulocyte volume and reticulocyte haemoglobin cellular content for normocytic, macrocytic and microcytic cases by automated method in study cases

	Microcytic (±SD), *n*=177	Normocytic (±SD), *n*=93	Macrocytic (±SD), *n*=30	*F*-test (*P*)
Mean IRF	0.14±0.08	0.15±0.11	0.23±0.17	7.78 (0.001)
Mean CRC	1.65±3.14	1.52±1.14	1.49±1.25	0.10 (0.89)
Mean MRV	87.73±21.63	111.16±24.44	128.26±26.78	58.11 (0.0001)
Mean RHCC	24.64±6.58	32.25±7.90	38.58±8.37	67.35 (0.0001)

SD=Standard deviation, IRF=Immature reticulocyte fraction, CRC=Corrected reticulocyte count, MRV=Mean reticulocyte volume, RHCC=Reticulocyte haemoglobin cellular content

Table 4: Correlation between manual and automated method of reticulocyte count by Pearson's correlation coefficient

	Mean	Standard deviation	*n*	Correlation	*P**
Manual method	4.3575	4.05398	300	0.985	0.0001
Automated method	4.1841	3.87314	300		

*Pearson's correlation coefficient test

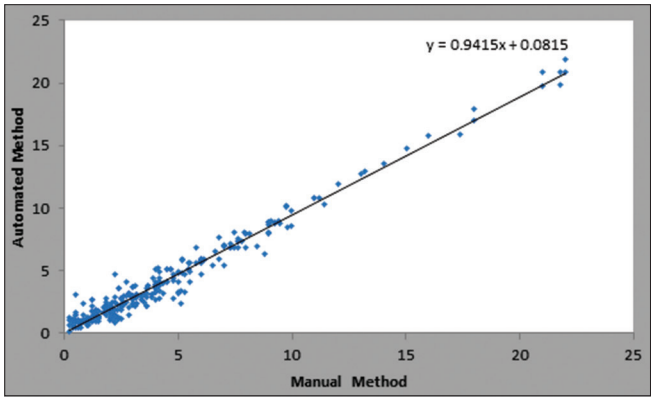

Figure 1: Correlation between manual and automated method of reticulocyte count

showed varied results in the literature. Osgood *et al.*[12] found no significant difference in average RC percentage in the age group of 4–13 years while; Jain *P et al.*[13] found a significant decrease in the elderly group. Bukhari and Zafar[14] found a significant difference in RC in (<27 days) age group in their study on infants ($P < 0.05$). Tarallo *P et al.*[10] found no statistical difference between boys and girls aged 4–19 years. However, it was significantly higher in men than in women over 20 years of age.[10]

The highest mean RC was observed in macrocytic anemia by both automated and manual methods followed by microcytic anemia in males in our study. The lowest mean RC was seen in microcytic anemia in female patients [Table 2]. The literature did not show such an association between the morphological type of anemia and mean RC. This study was mainly focused on finding out the difference between the manual and automated methods of reticulocyte counting. No statistically significant difference was observed between these two methods in microcytic, normocytic and macrocytic anemia. However, considering that most cases of macrocytic anemia will be having megaloblastic or hemolytic etiology,[15,16] it is expected that there will be reticulocytosis because of hemolysis and subsequent erythroid production. While, in females, the most common cause for not so significant increase in RC is iron deficiency anemia and as expected the mean RC was lowest in microcytic anemia in the present study. The most important cause of microcytic anemia is iron-deficiency anemia.[17] All these above findings indicate that there is no significant difference between mean RC obtained by automated and manual methods by age, sex, and morphology of anemia.

Regarding reticulocyte indices, we observed that the mean IRF was higher in macrocytic anemia as compared to microcytic and normocytic anemia ($P = 0.001$) [Table 3].

Our findings are consistent with Lacombe *et al.*,[18] Sindhu *et al.*[19] and Sunkara and Kotta,[20] all of which found a highly significant difference in IRF values. Rastogi *et al.*[21] found that a significant difference in the values of CRC obtained by manual versus automated method. The findings of our study showed that there was not much difference in manual and automated counts in both controls and cases, and the difference was also not statistically significant ($P = 0.89$). The reported differences between manual and automated RC may reflect on many variations in the staining technique, use of dye, and reliability by the observers. Furthermore, the deviation in the study by Rastogi *et al.*[21] was huge and it varied from minimum 2.2%–211%. In our study, we observed that MRV was higher in macrocytic anemia as compared to microcytic anemia ($P = 0.0001$). Our findings are consistent with Butthep *et al.*[22] who found a highly significant decrease in MRV in iron deficiency anemia patients versus normal (MRV = 95.89 ± 8.57 FL, $P \leq 0.0001$). Hence, it adds to the peripheral smear observation that the size of the reticulocytes observed in iron deficiency anemia is smaller than macrocytic anemia. We also found that RHCC was higher in macrocytic anemia as compared to microcytic anemia ($P = 0.0001$). Our findings are consistent with Butthep *et al.*,[22] Mast *et al.*,[23] Ceylan *et al.*,[24] and Ageeli *et al.*[25] ($P < 0.001$).

In this study, a significant positive correlation was found between manual and automated RC method by using Pearson's correlation coefficient (r) ($r = 0.985$, $P = 0.0001$) [Table 4 and Figure 1]. Our study is consistent

[Downloaded free from http://www.ijhonline.org on Wednesday, December 16, 2020, IP: 109.224.55.20]

with the studies of Lacombe *et al.*,[18] Simionatto *et al.*[26] and Ali *et al.*[27] who found a high degree of correlation and an excellent agreement between the two methods.

Conclusion

We conclude that there was no significant difference between automated and manual methods for reticulocyte counting in any gender for microcytic, normocytic, or macrocytic patients.

However, the manual method may be preferred as it is cost-effective; yet, it is laborious, time-consuming, need efficient technique, not suitable for heavy loaded laboratories and may be suitable for under-resourced laboratories. However, the automated method is preferred as it is fast, highly precise and it is mandatory for certain diseases where reticulocyte parameters are required as a statistically significant difference was found among the different parameters such as IRF, MRV, and RHCC.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Quigley JG, Means RT, Glader B. The Birth, Life, and Death of Red Blood Cells: Erythropoiesis, The Mature Red Blood Cell, and Cell Destruction. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, *et al*., editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2014. p. 83-124.
2. Patel K, Patel SM. Comparison of automated flowcytometric reticulocyte analysis with manual reticulocyte count. Int J Res Med Sci 2019;7:3825-32.
3. Yesmin MS, Sultana T, Roy CK, Rahman MQ, Ahmed AN. Reticulocyte parameter analysis in the automated haematology analyzer used in the laboratories. Bangladesh J Med 2010;21:80-3.
4. Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. Clin Chem Lab Med 2010;48:1369-80.
5. Viana KA, Assis O, Filho M, Avelar RS, Marquete D, Avelar V, *et al*. Reticulocyte count : Comparison among methods. Jr Bras Patho Med Lab 2014;50:339-45.
6. Davis BH, Bigelow NC. Automated reticulocyte analysis. Clinical practice and associated new parameters. Hematol Oncol Clin North Am 1994;8:617-30.
7. Torino AB, Gilberti Mde F, da Costa E, de Lima GA, Grotto HZ. Evaluation of erythrocyte and reticulocyte parameters as indicative of iron deficiency in patients with anemia of chronic disease. Rev Bras Hematol Hemoter 2015;37:77-81.
8. Briggs C, Bain BJ. Basic Haematological Techniques. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. 12th ed. Edinburgh: Elsevier Churchill Livingstone; 2017. p. 23-56.
9. Tefferi A, Hanson CA, Inwards DJ. How to interpret and pursue an abnormal complete blood cell count in adults. Mayo Clin Proc 2005;80:923-36.
10. Tarallo P, Humbert JC, Mahassen P, Fournier B, Henny J. Reticulocytes: biological variations and reference limits. Eur J Haematol 1994;53:11-5.
11. Okoroiwu IL, Obeagu, Ifeanyi E, Elemchukwu, Queen, Gloria DI. Reticulocyte count in healthy male and female students of Imo State University, Owerri. Int J Adv Res BiolSci 2015;2:131-3.
12. Osgood EE, Baker RL, Wilhelm MM. Reticulocyte counts in healthy children. Am J Clin Pathol 1934;4:292-6.
13. Jain P, Jain R, Shah C, Trivedi RS, Jain AK, Jindal M, *et al*. A prospective study for comparison of hematological parameters in healthy young adult and elderly age group subjects. Natl J Integr Res Med 2013;4:78-84.
14. Bukhari KT, Zafar H. Reference values of reticulocyte counts in five age groups of healthy infants at Rawalpindi, Pakistan. J Pak Med Assoc 2013;63:1108-11.
15. Colon-Otero G, Menke D, Hook CC. A practical approach to the differential diagnosis and evaluation of the adult patient with macrocytic anemia. Med Clin North Am 1992;76:581-97.
16. Razzak M, Begum N, Hossian D. Red blood cell fragility and reticulocyte count In hemolytic anemic patients with and without G-6PD enzyme deficiency. Banganbandhu Sheikh Mujib Med Univ J 2003;3:23-6.
17. Sadeghian M, Fatourechi A, Lesanpezeshki M, Ahmadnezhad E. Prevalence of anemia and correlated factors in the reproductive age women in rural areas of Tabas. J Family Reprod Health 2013;7:139-44.
18. Lacombe F, Lacoste L, Vial JP, Briais A, Reiffers J, Boisseau MR, *et al*. Automated reticulocyte counting and immature reticulocyte fraction measurement. Comparison of ABX PENTRA 120 Retic, Sysmex R-2000, flow cytometry, and manual counts. Am J Clin Pathol 1999;112:677-86.
19. Sindhu R, Behera SK, Mishra DP. Role of immature reticulocyte fraction in evaluation of aplastic anemia in cases of pancytopenia. Indian J Basic Appl Med Res 2016;5:619-24.
20. Sunkara A, Kotta DR. Evaluation of red cell indices and reticulocyte maturity indices including reticulocyte haemoglobin concentration in iron deficiency anaemia in adult female population. J Evid Based Med Healthc 2016;3:5315-8.
21. Rastogi S, Singh A, Chhabra P. Automated corrected reticulocyte count superiority above manual methods. Sch J Appl Med Sci 2016;4:1177-9.
22. Butthep P, Wisedpanichkij R, Jindadamrongwech S, Kaewkethong P, Pattamakom S, Sila-Asna M, *et al*. Reticulocyte analysis in iron deficiency anemia and hemolytic anemia. J Med Assoc Thai 2000;83 Suppl 1:S114-22.
23. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood 2002;99:1489-91.
24. Ceylan C, Miskioğlu M, Colak H, Kiliççioğlu B, Ozdemir 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.
25. Ageeli AA, Algahtani FH, Alsaeed AH. Reticulocyte hemoglobin content and iron deficiency: A retrospective study in adults. Genet Test Mol Biomarkers 2013;17:278-83.
26. Simionatto M, Paula JP, Nascimento AJ, Leonart MSS, Cicchetti D. Analysis of manual reticulocyte counts in the clinical laboratories of ponta-grossa and campos gerais, PR, Brazil. Rev Bras Hematol Hemoter 2009;31:315-20.
27. Ali AF, Moiz B, Omer S. Is manual reticulocyte count a reliable option for under resourced countries? J Pak Med Assoc 2010;60:892-6.