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Determining the Correlation Between Automated vs. Manual Method for Reticulocyte Estimation

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Abstract

Objective: To determine the correlation between automated and manual methods for the determination of reticulocyte (Retic) count in symptomatic patients.
Methodology: This cross-sectional study was conducted at Rehman Medical Institute, Peshawar, from November 2021 to April 2022. A total of 384 patients of both genders and all ages were enrolled. Anemic patients with high Retic counts who were referred to RMI for reticulocyte (RC) examination were included. Samples that were improperly proportioned with anticoagulants, lipemic, hyperbilirubinemic, or hemolyzed were excluded. The reticulocyte counts of all samples were analyzed using both manual methods and an automated analyzer (Sysmex XN analyzer), with evaluations performed by two different hematologists to eliminate observer bias.
Results: No significant deviation was observed between manual and automated reticulocyte counts in male or female specimens ($P > 0.05$). Similarly, there was no significant difference in average reticulocyte counts among adults, children, and infants ($P > 0.05$). However, a noticeable difference in mean manual and automated RC was observed across different types of anemia, including microcytic, normocytic, and macrocytic anemia. Additionally, the mean reticulocyte hemoglobin cellular content, reticulocyte volume, and immature reticulocyte fraction showed significant differences across all specimens ($P < 0.001$).
Conclusion: There were no significant deviations in reticulocyte counts between the manual and automated methods. The manual method is preferred for its cost-effectiveness, while the automated approach is recommended for its efficiency and accuracy.
Keywords: Manual reticulocyte count, Automated reticulocyte count, Anemia.

Introduction

Bone marrow releases its premature erythrocytes in peripheral blood which are known as reticulocytes. They therefore serve as a trustworthy indicator of recent erythrocyte formation in the bone marrow.[1] In the bone marrow, nucleated erythroid precursor cells develop and mature under typical conditions in about 24-72 hours. The newborn erythrocytes (also known as reticulocytes) are released into the peripheral circulation after their nucleus is excreted from the cell cytoplasm, where they float for 24-48 hours before maturing into erythrocytes.[2]

An accurate evaluation of blood is the initial phase for assessing hematological activity and diagnosing associated disorders.[3] To determine the level of erythropoietic function within the bone marrow, the reticulocyte count has developed as one of the fundamental but essential tests in clinical hematology.[4] Reticulocytes can be detected via manual reticulocyte counting (particular staining) or by automated reticulocyte counting (analysis of their remainder RNA).[2] This count offers a preliminary evaluation of anemia.[5] In case of hemolytic anemias and acute bleeding, or any other hemorrhagic conditions, monitoring patient response to the Vitamin B12, iron, and folic acid treatment can be extremely valuable for both prognostic and diagnostic purposes.[6]

The conventional technique for reticulocyte enumeration was established in the 1940s and involves manually counting reticulocytes for RNA through light microscopy using supravital dyes. Manual counting is relatively inexpensive and simple, but, it has some drawbacks and restrictions, including poor accuracy, incorrect blood films, an extended analysis duration, and poor stain efficiency. [7] With advancements in technology, clinical laboratories are now using automated reticulocyte counting techniques pioneered during the previous decade.[8]Through ongoing procedures, updated software, and advanced reagents, automated reticulocyte counting offers higher accuracy for the RC. It is a quick and straightforward examination that helps with sensitive evaluation and personalized tracking of anemic individuals.[9] It can reveal details about specific cell traits,

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including the hemoglobin content of reticulocytes, and that of the mature erythrocytes, the proportions of hypochromic and microcytic cells, the mRNA content, and cellular indices like volume, level, and content of hemoglobin. These unique factors can all be used for interpretation accordingly, especially when making a particular anemia diagnosis.[10]

Methodology

This cross-sectional research was conducted at Rehman Medical Institute, Peshawar, from. Rehman Medical Institute (RMI)research ethical committee authorized this study vide the letter number RMI/RMI-REC/Approval/130 dated 20th Oct 2021. Prior to the investigation, research participants were provided with a detailed explanation of the procedure, and were asked for their informed consent for inclusion in the study. The WHO calculator was used to determine the sample size, taking a confidence interval of 95%, with a 5% margin of error, a Z score of 1.96, and a population proportion of 0.5. The sample size came out to be 384. Moreover, a non-probability and consecutive technique was employed for the sampling process.

Inclusion Criteria: In this investigation, all the subjects that were anemic and referred to RMI Lab for the retic count were included. This study employed both male and female subjects of all ages having higher retic count. The TLC and platelet count of all these individuals were in normal ranges.

Exclusion Criteria: All the lipemic, hyperbilirubinemic, and hemolyzed samples were excluded from this study after visual assessment. Blood samples that were not in proportion with anticoagulants were also excluded from this research.

After obtaining the informed consent, a 3ml blood sample was collected from each participant by venipuncture into Tri potassium Ethylene diamine tetra-Acetic acid (K_3-EDTA) vacuum tubes. The samples were blended thoroughly at the time of venipuncture and again just prior to beginning analysis. All these samples were stored at optimum room temperature. Reticulocyte staining, according to ICSH's specifications, was done to perform the manual count, by taking 2 drops of filtered new methylene blue solution and mixing it with an equal amount of blood. The mixture was kept at room temperature (37°C) for about 15 minutes. After mixing it gently, a thin smear was prepared on a glass slide with a small drop of the mixture on it. After air drying the mixture, the slide was examined under the microscope using an oil immersion objective. Retic count was performed by two hematologists independently to eliminate observer bias. An automated retic count was also done by the Sysmex XN analyzer. It employs fluorescent dyes which bind to the RNA content of reticulocytes and analyses it by flow cytometry. The differential parameters of retic counts studied in this investigation involve immature reticulocyte fraction (IRF), mean reticulocyte volume (MRV), reticulocyte hemoglobin cellular content (RHCC), and reticulocyte index (RI) or corrected reticulocyte count (CRC). The reference values of these parameters encompass the following values: hemoglobin (12-16 g/dL) retic count (0.5-2.5%), immature reticulocyte fraction (2.3-15.9%), mean reticulocyte volume (93.1-114.8 fL), reticulocyte hemoglobin cellular content (28-36 pg), and corrected reticulocyte count (0.5-1.5%).

Statistical Package for Social Sciences (SPSS) version 23.0 was used for data analysis. Descriptive statistics including the mean and standard deviation were determined for continuous variables. Z-test, F-test, and Pearson's correlation coefficient test were applied to find the difference between the two means, and $P < 0.05$ was considered significant.

Results

A total of 384 subjects of all ages were employed in this research. Out of the total subjects, 224 (58.3%) were male and 160 (41.7%) were females. The mean age of male subjects was 29.30±11.44 and of female subjects was 27.40±10.20. We divided the subjects into 5 different age groups. The mean automated RC for males was 4.69±2.5943 and it was 4.80±2.3756 by the manual approach. The mean automatic RC for females was 4.74±1.7438 while the mean manual RC was 4.876±1.6507. By using the z-test, it was determined that there was no statistically significant difference between the automated and manual count for either the male ($P < 0.001$) or female patients. [Table-I upper half]. The mean automated RC among age group 1 (0-15 years) was 4.30±1.9346 and it was 4.51±1.8104 by manual

method. In the case of group 2 (15-30 years), the mean automated RC was 4.777±2.6482 and 4.85±2.4388 by manual method. For group 3 (31-45 years), the mean automated RC was 4.72±1.6102 and 4.85±1.5171 by manual method. For age group 4 (46-60 years), the mean automated RC and the mean manual RC were 4.41±1.2559 and 4.60±1.1717 respectively. In the case of age group 5 (>60 years), the mean automated RC was 6.59±3.4641 and 6.85±2.7135 by manual method. A z-test for the difference between the two means was applied. In the lower half of Table-I, no statistically significant difference was discovered in the average RC (performed by both manual and automated approaches) of all the age groups (P < 0.001) (Table I; lower half).

Table I: Comparison of mean RC (by both manual and automated procedures) of both males and females(n=384) based on their age.

Gender	Automated Reticulocyte Count (Mean±SD)	Manual Reticulocyte Count (Mean±SD)	z-test (p-value)
Male (n=224)	4.69±2.5943	4.80±2.3756	<0.001
Female (n=160)	4.74±1.7448	4.876±1.6507	
Comparison of Reticulocyte count according to Age			
0 to 15 years	4.30±1.9346	4.51±1.8104	<0.001
16 to 30 years	4.777±2.6482	4.85±2.4388	
31 to 45 years	4.72±1.6102	4.85±1.5171	
46 to 60 years	4.41±1.2559	4.60±1.1717	
>60 years	6.59±3.4641	6.85±2.7135	

Table II: Mean reticulocyte count in study subjects based on morphologic anemia categorization. (n=384)

Anemia	Automated RC (Mean±SD)	Manual RC (Mean±SD)	z-test (p-value)
Microcytic Anemia (n=52)	4.42±1.4157	4.75±1.5040	<0.001
Normocytic Anemia (n=278)	4.73±2.5221	4.80±2.3079	
Macrocytic Anemia (n=54)	4.88±1.4728	5.04±1.3624	

As shown in Table II, when patients were categorized into groups according to the morphologic classification of anemia into microcytic anemia, normocytic anemia, and macrocytic anemia, the discrepancy between mean manual and automated RC was statistically significant in each group.

As shown in Table III, the mean IRF, mean CRC, mean MRV, and mean RHCC have been found to be statistically significant across all kinds of anemia when comparison was made on the basis of reticulocyte indices (P < 0.001).

As demonstrated in Table IV and Figure I, a substantial positive connection between the manual and automated RC approaches was discovered using Pearson's correlation coefficient (r = 0.968, P < 0.001).

Table IV: Comparison of the reticulocyte count using manual and automated techniques based on Pearson's correlation coefficient (n=384)

	Mean±SD	Correlation r^2	p-value
Automated Method	4.71±2.2767	0.968	<0.001
Manual Method	4.83±2.1020		

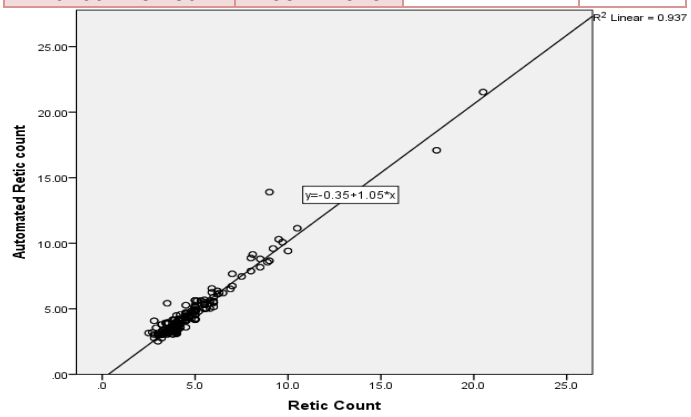

Figure-I: Correlation between manual and automated methods of reticulocyte count

Discussion

Reticulocyte count is a pivotal indicator of bone marrow erythropoietic function.[11] Reticulocyte estimation has diagnostic and prognostic significance in the evaluation of anemia, acute hemorrhage, response to hematinic, as well as, after chemotherapy and in the assessment of

Table III: Comparison of subjects', automated mean immature reticulocyte fraction (IRF), mean corrected reticulocyte count(CRC), mean reticulocyte volume(MRV), and reticulocyte hemoglobin cellular content (RHCC) for different anemias including normocytic, macrocytic, and microcytic (n=384)

Parameters	Microcytic (Mean±SD)	Normocytic (Mean±SD)	Macrocytic (Mean±SD)	F-test (p-value)
IRF	23.48±9.5405	21.84±10.34	30.04±11.23	14.15 (0.000)
CRC	2.56±0.7626	3.31±1.642	3.87±0.9657	10.48 (0.000)
MRV	87.92±2.0328	108.92±5.8415	128.99±7.0202	693.43 (0.000)
RHCC	22.20±3.2104	27.50±3.34	30.19±3.82	77.98 (0.000)

stem cell engraftment after bone marrow transplantation.[12] Its estimation can be performed by both manual and Automated techniques.[13] Both of these techniques have their advantages and disadvantages.[14]

Based on their gender, age, and different types of anemias, participants in the current study were classified into a number of groups. The samples were all subjected to manual and automated analyses for different parameters, including IRF, CRC, MRV, and RHCC. According to the current study, the mean reticulocyte count estimated by both procedures in males and females did not differ significantly. There were also no significant differences in the RC determined by manual and automated methods in various age groups. However, there is controversy in existing literature regarding the correlation between the two methods.

Just like the present study, a study by Gorte TR *et al,* showed comparable results. According to this study, there were no significant differences in the mean RC determined by manual technique (p=0.61) and the mean RC determined by automated analyzer (p=0.77). Additionally, there were also no as such differences between mean RC of participants of various ages including newborns (p=0.71), children (p=0.59), and adults (p=0.66) in terms of significance.[15]

The present research showed consistent results with another research by Lacombe F et al.[16] This investigation compared different techniques for RC including Sysmex R-2000, Manual counts, ABX PENTRA 120 Retic, and Flow cytometry. The findings demonstrated a substantial correlation between these approaches and a lack of significant differences in their outcomes for reticulocyte count.[16] An investigation by Ali AF et al, also suggested that there are no substantial differences in RC conducted by manual procedure and by automated analyzer. [17]

A study by Patel K et al showed contrasting results to the present study.[18] According to this research, the automated analyzer hematological analyzer has a minimal carryover and great accuracy and linearity. However, case-by-case percent variations between manual and automated CRC and RC were influenced substantially when evaluating the manual and automated RC techniques (correlation coefficient r-0.865). The run precision of both techniques varied greatly. The average difference among repeated measurements of manual and automated RC (5%) in 150 specimens was 0.3 and 0.01, but for RC>5%, it was 6.3 and 0.15. This study discovered that the automated approach is more accurate than the manual one.[18]

Another investigation by Rastogi Set al, demonstrated the superiority of automated analyzers for CRC. [19] The values of CRC varied substantially among both automated and manual methods. This variation ranged from a minimal 2.2-211%, and the mean percent deviation was 34.1%. The results of this investigation proposed that the automated RC count was fast, and error-free as compared to manual procedure.[19]

As per the previously available literature, there is a controversy regarding the correlation between the manual and automated approaches for RC. Furthermore, the lack of locally published literature necessitated the present investigation.

Conclusion

This research demonstrated no significant deviations in reticulocyte count performed either by manual or by automated analyzer in individuals with different types of anemias including normocytic, microcytic, and macrocytic regardless of their age and gender. The manual approach, however, could be preferred since it is less expensive; yet, it is tedious, and time-consuming, and is impractical for laboratories with substantial workloads, and might be ideal for laboratories with limited resources. However, the automated approach is recommended since it is quick, extremely accurate, and essential for several conditions that demand reticulocyte parameters because a statistically substantial deviation was discovered among several parameters, such as RHCC, IRF, and MRV.

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