Original Investigation | Özgün Araştırma

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To Evaluate Sensitivity of Microcyte% and Macrocyte% Parameters in Comparison with Peripheral Blood Smear in Normocytic Normochromic Anemia Patients

Normositik Normokromik Anemi Hastalarında Mikroset% ve Makroset% Parametrelerinin Periferik Kan Yayması ile Karşılaştırmalı Olarak Duyarlılığını Değerlendirmek

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ABSTRACT

Objective: To compare the sensitivity of microcyte and macrocyte percentage given by the automated cell counter with peripheral blood smear (PBS) in normocytic normochromic (NCNC) anaemia, and to set a normal range for microcyte and macrocyte% using the data from normal control samples.

Methods: It is a cross-sectional study; whole blood samples (EDTA vacutainer) were run on a six-part cell counter, and peripheral blood findings were entered into an Excel sheet. Inclusion criteria: Age more than 18 years and having NCNC anemia. Exclusion criteria: Patients with a history of recent blood transfusion or on haematinics.

Results: A total of 240 samples were studied. Research use only (RUO) microcyte% was 44.16%, RUO macrocyte% was 27.5%, and the mixed cell population group was 15.83%. Sensitivities of both research-use-only parameters were 100%, with specificities of 95% for RUO–microcyte% and 97.7% for RUO–macrocyte%, whereas diagnostic accuracy was 97.5% for RUO–microcyte% and 98.3% for RUO–macrocyte%.

CONCLUSION: Microcyte and macrocyte percentages provided by the machine are more sensitive, as they can screen millions of RBCs and are not dependent on the observer's skills, compared to PBS examination. Depending on the percentage of microcytic or macrocytic cells, we can recommend further confirmatory biochemical tests. Thus, we can identify a treatable underlying cause.

Keywords: Whole blood sample, automated cell counter, peripheral blood smear, microcyte% and macrocyte%

ÖZ

Amaç: Normositik normokromik (NCNC) anemide otomatik hücre sayacı tarafından verilen mikroset ve makroset yüzdesinin periferik kan yayması (PBS) ile duyarlılığını karşılaştırmak ve normal kontrol örneklerinden elde edilen verileri kullanarak mikroset ve makroset % için normal bir aralık belirlemek.

Yöntemler: Kesitsel bir çalışmadır; tam kan örnekleri (EDTA vakutainer) altı parçalı bir hücre sayacı üzerinde çalıştırıldı ve periferik kan bulguları bir Excel tablosuna kaydedildi. Dahil etme kriterleri: 18 yaşından büyük olmak ve NCNC anemisi bulunmak. Hariç tutma kriterleri: Yakın zamanda kan transfüzyonu geçirmiş veya hematiniks kullanan hastalar.

Bulgular: Toplamda 240 örnek incelendi. Araştırma amaçlı kullanım (RUO) mikro sit %44,16, RUO makro sit %27,5 ve karışık hücre popülasyonu grubu %15.83 idi. Her iki araştırma amaçlı parametrenin de duyarlılıkları %100 olup, RUO–mikrosit % için özgüllük %95 ve RUO–makrosit % için %97.7 iken, tanısal doğruluk RUO–mikrosit % için %97,5 ve RUO–makrosit % için %98,3'tür.

Sonuç: Makinenin sağladığı mikroasit ve makroasit yüzdeleri, milyonlarca RBC'yi tarayabildiği ve gözlemcinin becerilerine bağlı olmadığı için PBS incelemesine kıyasla daha hassastır. Mikrositik veya makrositik hücrelerin yüzdesine bağlı olarak, daha fazla doğrulayıcı biyokimyasal testler önerebiliriz. Böylece, tedavi edilebilir bir altta yatan nedeni belirleyebiliriz.

Anahtar Sözcükler: Tam kan örneği, otomatik hücre sayacı, periferik kan yayması, % mikrosit ve % makrosit

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INTRODUCTION

According to the World Health Organization, anaemia is a condition in which either the number of red blood cells (RBCs) or the Hb concentration within them is lower than normal. Anemia is defined as a Hb level <12.0 g/dL in women and <13.0 g/dL in men (1). The two common methods of classification of anaemia are morphologic and pathophysiologic. In the morphological classification, anaemia is subdivided into three major groups based on blood indices, particularly mean corpuscular volume (MCV) (2). According to the RBC morphology system for classifying anemia, there are three basic divisions in the morphologic classification:

a. Microcytic–MCV <80 fL

b. Macrocytic–MCV >100 fL

c. Normocytic–MCV 80–100 fL

MCV is the mean volume of RBCs in a sample. It may serve as a useful parameter to classify anemias based on RBC size (3). The MCV is the average volume of RBCs; it was previously calculated from the hematocrit (volume of packed red cells) and the erythrocyte count. Modern hematology analyzers perform cell sizing; therefore, the MCV is a measured parameter (4).

Since the MCV is the mean volume of RBCs, it does not reflect variation in RBC size within the sample. A sample with a normal MCV may have groups of smaller and/or larger RBCs within the total counted population. The excessive variation in RBC size is known as anisocytosis. It is assessed by a pathologist by reviewing a peripheral blood smear (PBS).

Thus, MCV must always be interpreted in conjunction with a review of the PBS, RDW, RBC graph, and reticulocyte count.

Automated cell counter Mindray BC-6000/BC-6000 Plus has research use only (RUO) parameters named microcyte% and macrocyte% (5). Microcyte% and fraction: in this machine, all cells in the RBC chamber with volumes less than 60 fL are counted. Macrocyte% and fraction—in this machine, all cells in the RBC chamber with volumes greater than 120 fL are counted (6). These indices are rapidly obtained, inexpensive, and can be effective as preliminary screening tools (5). On PBS examination, anisocytosis can be detected, but there are limitations for the observer. In PBS, we can screen up to a thousand cells. The microcyte and macrocyte% (RUO parameters) provided by the machine (Mindray BC6000) are available free of charge and can be used as screening tests to determine microcytic and macrocytic cell percentages. It is more sensitive than PBS examination, as it can screen millions of RBCs and is not dependent on the observer's skills. Depending on the percentage of microcyte or macrocyte cells, we can advise further confirmatory biochemical tests. Thus, we can reach a treatable underlying cause.

These parameters will be useful in cases of falsely normal MCV resulting from two distinct cell populations. Normocytic normochromic (NCNC) anemia causes are anemia of chronic disease (ACD), acute blood loss, chronic renal failure, hemolytic anemia, and mixed nutritional deficiency, etc. The pathogenesis, epidemiology, and clinical characteristics of NCNC anemia of unknown cause are not well established (7).

The study was conducted with the overall aim of evaluating the sensitivity of microcyte and macrocyte percentages given by the cell counter compared with PBS in NCNC anaemia.

Aims and Objectives

1. To compare sensitivity of microcyte and macrocyte percentage given on Mindray BC6000 with morphological findings on PBS in NCNC anaemic patients.

2. To set normal range for microcyte and macrocyte% (RUO parameter) using the data from 120 normal control samples.

MATERIALS AND METHODS

A cross-sectional study was conducted at the central clinical laboratory, Department of Pathology, in a tertiary care hospital. A sample size of 120 was calculated at a precision of 0.1 and a 95% confidence interval using the following formula (8).

Formula used: $n = z^2S (1-S) / d^2$, where z^2 is the area under the curve for the confidence level, S is the sensitivity, and d is the absolute precision. n = (a + b + c + d).

A consecutive and purposive sampling procedure was used. Each of the 120 samples was studied for the anaemic and control groups. Patients above 18 years of age with Hb <12 gm% in females and <13 gm% in males with normal MCV (80–100 fL) were included. The control group includes individuals above 18 years with normal MCV & normal haemoglobin for gender. The control group includes individuals older than 18 years with normal MCV and normal haemoglobin for their gender. Patients who had a recent blood transfusion or were on haematinic supplements were excluded from the study. Venous blood samples were drawn into vacutainers containing K2-EDTA anticoagulant. Samples were stored at ambient temperature and processed within 6 hours of collection. Data obtained from analysis of an EDTA-anticoagulated whole-blood sample on a six-part differential cell counter (Mindray BC 6000), together with peripheral blood findings, were entered into an Excel sheet. The analyzer was calibrated, controlled, and maintained according to the manufacturer's recommendations. The variables studied were MCV, PBS, microcyte% and macrocyte% (RUO parameter).

Maharashtra Institute of Medical Education and Research (MAEER MIT PUNE), Dr. Bhausaheb Sardesai Talegaon Rural Hospital Ethics Committee approval was obtained (EIC reference no MIMER/IEC/1832/09/2021, dated 21.09.2021). Written informed consent was obtained from the study participants. No additional cost was incurred by the study participants.

Statistical Analysis

Statistical analysis: mean, range, and standard deviation were calculated for the control group using IBM SPSS Software version 26.

Using the mean and standard deviation, cut-offs were obtained from the control group, and data from the study group were analyzed and correlated with peripheral smear findings.

RESULTS

A total of 240 samples were included in the study, of which 120 were in the NCNC anemia group (shown in Figure 1) and 120 were in the control group with normal Hb and MCV.

The gender-wise distribution shown in Table 1 indicates an equal distribution of samples between males and females.

Reference ranges were obtained for the parameters MCV, RUO–microcyte%, and RUO–macrocyte% using data from the control group, as depicted in Table 2.

Table 3 shows the study-group analysis from the automated cell counter, reporting RUO–microcyte% as 44.16%, RUO–macrocyte% as 27.5%, and 15.83% in the mixed cell population group.

Table 4 compares all three groups assessed with the automated cell counter and PBS and shows comparable results for RUO–microcyte% and RUO–macrocyte%.

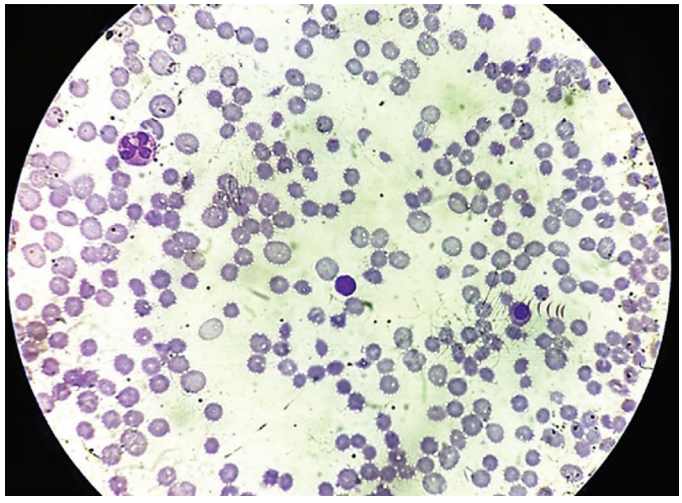


Figure 1. Microphotograph (40x) showing macrocytic and microcytic population of cells on peripheral blood smear.

Table 1. Gender wise distribution of control and study group.

	Control group (normal hemoglobin)	Study group (anemic patients)
Males	64 (53.33%)	65 (54.16%)
Females	56 (46.67%)	55 (45.84%)

Table 2. Reference range of parameters using data from control group.

Parametres	Mean	SD	Reference range, (mean ± SD)
MCV-fL	87.20	8.45	87 ± 16.9
RUO-microcyte%	1.16	1.09	0–3.34
RUO-macrocyte%	2.55	1.51	0–5.57

SD: Standard deviation, RUO: Research use only, MVC: Mean corpuscular volume.

Table 3. RUO percentage values of automated cell counter in study group.

Parameters	Total no of patients	Percentage
RUO-microcyte%	53/120	44.16
RUO-macrocyte%	33/120	27.5
Total	86/120	71.66
Mixed cell population	19/120	15.83

RUO: Research use only.

Table 5 shows 100% sensitivity for both RUO parameters, 95% specificity for RUO–microcyte

%, and 97.7% for RUO–Macrocyte%, whereas the Diagnostic accuracy of RUO–microcyte% is 97.5% and 98.3% for RUO–macrocyte%.

DISCUSSION

With advances in technology over the last 10–15 years, additional RBC parameters have been introduced on automated hematology analyzers. Although many of these advanced indices are currently available on hematology analyzers as RUO parameters, there is increasing evidence of their clinical importance in the diagnosis and in the assessment of the severity of anemia, as well as in monitoring treatment response. These advanced RBC parameters are: mean cellular Hb concentration, calculated cellular Hb, Hb distribution width, percent microcytosis (%MIC), percent macrocytosis (%MAC), percent hypochromia, and percent hyperchromia. The present study focused on %MIC and %MAC.

$$\text{RUO–microcyte\%} = \frac{\text{The number of RBC smaller than 60 fL}}{\text{Total number of RBC}} \times 100\%$$

$$\text{RUO–macrocyte\%} = \frac{\text{The number of RBC larger than 120 fL}}{\text{Total number of RBC}} \times 100\%$$

The percent microcytic RBCs (%MIC) represents the proportion of microcytic RBCs in a sample. The %MIC is derived from the RBC volume distribution histogram and usually includes RBCs with volumes below 60 fL, as shown in Figure 2 (9,10). %MIC can be used in combination with other RBC parameters to help differentiate between iron deficiency anemia (IDA) and thalassemia (11). The percent macrocytic RBC (%MAC) is the proportion of macrocytic RBCs in a sample, reported as a percentage. The %MAC is derived from the RBC volume distribution histogram and usually includes RBCs with volumes above 120 fL. The frequency of microcytic and macrocytic RBCs can be assessed by visual estimation on a stained

Table 4. Comparison of RUO percentage values given by automated cell counter and peripheral blood smear in study group.

Parameters	Automated cell counter	Peripheral smear (gold standard)	Comparison percentage
RUO-microcyte%	53 (44.16%)	50 (41.66%)	94.33%
RUO-macrocyte%	33 (27.5%)	31 (25.83%)	93.93%
Mixed cell population	19 (15.83%)	16 (13.33%)	84.21%

RUO: Research use only.

Table 5. Sensitivity, specificity and diagnostic accuracy of RUO parameters compared to peripheral smear findings.

	RUO-microcyte%	RUO-macrocyte%
Sensitivity	100%	100%
Specificity	95%	97.7%
Diagnostic accuracy	97.5%	98.3%

RUO: Research use only.

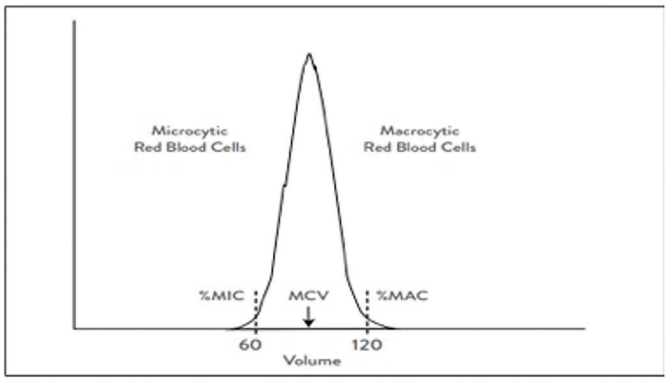


Figure 2. RBC distribution histogram depicting MIC% and MAC%.

MIC%: Percent microcytosis, MAC%: Percent macrocytosis, RBC: Red blood cell.

PBS. The %MIC and %MAC from automated hematology analyzers, however, offer a more precise quantitation. A low degree of anisocytosis cannot be easily diagnosed on a peripheral smear, as a trained and skilled person is required for such interpretation

On PBS, we can screen only a limited number of cells, up to thousands. The microcyte and macrocyte percentages provided by the machine are available at no cost and can be used as screening measures to determine the proportions of microcytic and macrocytic cells. It is more sensitive as it can screen millions of RBC and is not dependent on the observer's skills compared to PBS examination.

NCNC anemia is caused by many conditions. Depending on the percentage of microcytic or macrocytic cells, we recommend further confirmatory biochemical testing. Thus, we can identify a treatable underlying cause, such as nutritional deficiency, and avoid further invasive procedures.

Barton JC conducted a study quantifying microcyte and macrocyte percentages in archived RBC volume histogram images. They used a tool to analyse the RBC graph and calculate the percentage, whereas the present study calculates the percentage using the machine itself (6).

The study conducted by Urrechaga (12) evaluated the discriminant index value of the ratio of % microcytic cells to % hypochromic cells (i.e., M/H ratio) in the differential diagnosis of microcytic anemia. The M/H ratio used by him is a useful parameter to differentiate between IDA and beta thalassemia trait; the latter shows a distinct pattern of microcytic and hypochromic cells that can be detected by this parameter.

There are no studies conducted on the utilisation of micro% and macro% in NCNC anemias.

ACD is the second most common cause of anemia after iron deficiency (13).

A study by Singh et al. (14) showed a 44.88% prevalence of normocytic anemia.

The pathophysiology involves dysregulation of iron metabolism due to chronic inflammation (13). Most patients with ACD present with normocytic, normochromic, or mildly hypochromic RBCs. The MCV is usually in the range of 75–82 fL, and the HGB is rarely less than 9.0 g/dL. MCH, MCHC, and RDW are within normal limits. Additionally, serum iron, transferrin saturation, and total iron-binding capacity may be decreased, but serum ferritin is normal or elevated (15).

Functional iron deficiency occurs when iron stores are transiently unable to meet the demands of increased erythropoiesis, usually during treatment with erythropoietin-stimulating agents (16).

Normocytic, normochromic anemia differs from other forms of anemia because the average size and Hb content of RBCs are typically within normal limits. RBCs typically appear similar to normal cells under microscopic examination; however, in some cases variations in size and shape may offset one another, resulting in mean values within the normal range (17). ACD is usually a mild-to-moderate, normocytic, normochromicanemia; it evolves over time to become hypochromic and, more rarely, microcytic. Less than 25% of NCNC anemia cases progress to microcytic hypochromic anemia; in such cases, the MCV is rarely less than 70 (18).

The dimorphic blood picture reveals a dual- cell population on PBS, whereas indices displayed by automated cell counters may indicate low, normal, or high MCV. Such a diagnosis cannot rely solely on results from an automated cell counter because this may lead to inaccuracies (19). Therefore, it is essential to examine all cell populations using PBS, and it can be better predicted using microcyte% and macrocyte% measured by an automated cell counter.

Iron-deficiency anemia in its early stages can appear as normocytic, normochromic anemia. Iron-deficient erythropoiesis is characterized by the production of RBCs with decreased Hb content, leading to a high percentage of hypochromic cells; these cells also tend to be more microcytic as depletion progresses. Because of their long lifespan, several cohorts of normocytic and microcytic RBCs coexist in the peripheral blood, leading to anisocytosis (20).

Mohammed and Mahmood (21) in their research, concluded that normochromic anemia is the most prevalent anemia associated with renal disease. Anemia frequently occurs as a complication of various chronic illnesses, particularly chronic kidney disease; it is often referred to as anemia of renal disease and is associated with iron deficiency. This condition is typically linked to diminished quality of life and increased mortality and morbidity in patients (21). Thus, these new parameters, microcyte% and macrocyte%, can be helpful in such cases.

Study Limitations

A smaller sample size and confirmatory biochemical tests were not available in this scenario, but they should be used in combination with these parameters.

CONCLUSION

To conclude, this study helped us set cutoffs for microcyte% and macrocyte%: microcyte% greater than 3.34 and macrocyte% greater than 5.57 should be considered abnormal for the given population. Patients with elevated values, depending on the type of measurement, should be evaluated for iron deficiency, vitamin B12 deficiency, or both.

In resource-constrained conditions where a skilled pathologist is not available, RUO–Microcyte% and RUO–macrocyte% provided by the automated cell counter are more sensitive as they can screen

millions of RBC and are not dependent on observer skills compared to PBS examination.

Ethics

Ethics Committee Approval: Maharashtra Institute of Medical Education and Research (MAEER MIT PUNE), Dr. Bhausaheb Sardesai Talegaon Rural Hospital Ethics Committee approval was obtained (EIC reference no MIMER/IEC/1832/09/2021, dated 21.09.2021).

Informed Consent: Written informed consent was obtained from the study participants. No additional cost was incurred by the study participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.M.N., Concept: H.D., Design: H.D., Data Collection or Processing: S.M.N., V.D.P., Analysis or Interpretation: S.M.N., V.D.P., Literature Search: S.M.N., Writing: S.M.N.

Conflict of Interest: No conflict of interest was declared by the authors.

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