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Comparision of RBC size and mean corpuscular volume in automated peripheral smear system

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Abstract
Introduction: The MCV procedure is used to determine the average size of red blood cells as well as any anomalies. It is part of a routine check-up to detect any anomalies. Haematocrit values and RBC count are used to measure MCV. Despite the fact that the MCV is a predictor of RBC scale, the estimate relied solely on the PCV and RBC count. The aim of this research is to determine the relationship between MCV and RBC scale.
Materials and Methods: A total of 80 patients' blood samples were randomly obtained from an academic laboratory. To save the blood from clotting, anticoagulants are used. The RBC size is determined using a regular formula from the collected sample's peripheral smear. The automatic hemology analyser is used to extract MCV from the laboratory. The data were statistically analyzed.
Results and Conclusion: According to the analysis, a pathologist will evaluate macrocytic or microcytic RBCs without using an automatic analyzer and without scanning for and comparing the size of small lymphocytes by using the peripheral smear alone and comparing the MCV values. There is a need for a scale (in-built Vernier caliper) in the microscope, which is useful for RBC size measurement and also saves time. According to the findings, there is a statistically significant linear association between RBC size and MCV.

Keywords: RBC size, MCV, mean corpuscular volume, peripheral smear, automated machine, anemia

Introduction

A cellular part of the blood is red blood cells, also known as erythrocytes. There are millions of these in the circulation of vertebrates, giving the colour its distinctive appearance. A discocyte-shaped typical human RBC has a diameter of 7.5 to 8.7 m and a thickness of 1.7 to 2.2 m. The cytosol of RBC contains the haemoglobin molecules that are needed for gas transport within the body circulation. At 300 mOsmol/kg, the membrane controls the amount of cytosol in the intracellular RBC fluid, which averages 94 m3 (the normal unit osmol calculates osmotic pressure as osmols per kilogram [Osmol/kg]; milliosmol [mOsmol] is one-thousandth of one Osmol) [1]. The RBC cell membrane is made up of a phospholipid bilayer with a two-dimensional network of spectrin molecules below it. The discocyte morphology of stable RBC is the product of the combined properties of the spectrin molecules and the phospholipid bilayer. It provides the cell membrane with elastic and biorheological properties. The spectrin network or cytoskeleton provides the RBC with its shear elastic properties. The bilayer and spectrin network are linked by integral and peripheral proteins. Vertical interactions refer to associations that are involved in protein binding; horizontal interactions refer to binding that is involved in the two-dimensional spectrin network formation [2].

For certain RBC genetic diseases, disturbances to these vertical and horizontal connections result in modifications to the spectrin network density, which in turn induces changes in cell morphology, membrane fluctuations, and RBC deformability. The RBC has a one-of-a-kind potential to undergo repeated significant deformations. It is responsible for the migration of these cells through blood vessels as narrow as 2–3 m in diameter during circulation. The spectrin network's dynamic cytoskeleton remodeling has been shown to aid fluidity [3].

The biconcave shape of the human red blood cell (RBC) and its resulting deformability are important features of its biological role [4]. The primary purpose of the RBC and its hemoglobin is to transport oxygen from the gills or lungs to all body tissues, as well as carbon dioxide, a waste product of digestion, from the body tissues to the lungs, where it is

expelled. The abbreviation MCV stands for mean corpuscular volume. Red blood cells, white blood cells, and platelets are the three primary corpuscles (cells) of the circulating blood. MCV checks are used to determine the average size of red blood cells as well as any anomalies. A full blood count also includes a mean corpuscular blood examination. It is part of a routine check-up to detect any anomalies. The anomalies are used to determine whether certain illnesses, such as anemia (a blood disorder), vitamin deficiency, or any other medical illness, have occurred. The exam does not require any special training. This procedure is carried out by health care practitioners who use a thin needle to take a blood sample from a vein, normally in the arm. A small amount of blood would be gathered into a test tube or vial after the needle is inserted. Because of the action of the needle, there is a little pain when drawing blood. It normally takes less than five minutes to complete this task. In order to prevent contamination, the syringe must be sterilized. Anemia, especially iron deficiency anemia and thalassemia, may be caused by abnormalities in mean corpuscular value. Anemia is a disease in which the blood has less red blood cells than average. The most prevalent form of anemia is iron deficiency anemia. Thalassemia is an inherited blood disorder that can lead to serious anemia.

A reduction in mean corpuscular volume causes the aforementioned condition. Vitamin B12 deficiency, folic acid deficiency, another form of B vitamin deficiency, liver disease, and hypothyroidism can all be caused by a rise in mean corpuscular volume, or the size of RBC, above the average amount. Diet, exercise frequency, medications, a woman's menstrual cycle, and other factors may all influence the outcome. Erythrocyte volume homeostasis disorders are a diverse group of rare diseases with phenotypes ranging from dehydrated to overhydrated erythrocytes. The maintenance of cellular volume homeostasis is important for the erythrocyte's stable survival [5]. A typical characteristic of many hereditary anemias is a disruption of this homeostasis, which results in irregular RBC scale. Water and solute homeostasis in normal red cells is mediated by many mechanisms, with monovalent cation material mainly controlling cellular volume [6]. By successfully transferring sodium out of and potassium into the cell, the sodium-potassium ATPase pump (Na+K+ATPase) keeps the erythrocyte's intracellular low sodium, high potassium composition. When inward sodium leakage exceeds potassium leakage out, RBC swells; when potassium leakage out exceeds inward sodium leakage, RBC shrinks. The study of intracellular potassium, sodium and altered indices of erythrocyte hydration and total cation content, such as increased or decreased mean corpuscular hemoglobin concentration (MCHC) detects changes in membrane permeability. A mean corpuscular volume measurement can also be used to diagnose hereditary spherocytosis, an unusual blood condition in which mutations in red blood cells cause them to be formed like spheres and quickly break down. A high mean corpuscular volume (MCV) is linked to aging, diet, and substance dependence, among other things, and it's been used as an indicator of survival of chronically ill patients. The existence of the necessary "ingredients" (i.e., iron, folic acid, and vitamin B12), a normal working bone marrow, and erythropoietin for red cell stimulation are all required for normal RBC development. As a result, any lack of the above-mentioned variables causes a shift in MCV value. This research aims to assess and compare RBC size and mean corpuscular length, as well as their associated anomalies.

Materials and method

Form of blood collecting and sample selection after receiving their approval, a prospective study was performed on forty dipotassium EDTA blood samples obtained from randomly selected patients attending routine blood examinations at the Clinical Laboratory of a tertiary care hospital in South India. The aim of creating a smear is to protect the sample from getting lost during the staining process. The patient's name (or other identifiers), as well as the date and time of collection, are written on pre-cleaned slides. Blood films are created by putting a drop of blood on one end of a slide and spreading it out over the length of the slide using a spreader slide. This blood sample monolayer is collected. The drop is smeared gently and quickly with a wedge technique to leave a thin "feather" edge on which all cells, especially red blood cells, can be inspected individually. The smear-covered surface of the slide should not be touched. The blood collection sample size was about 80.

Calculation of RBC size

After that, the prepared peripheral smear is stained. For staining purposes, Leishman stain is used. Methylene blue and Eosin dye are mixed in an alcohol medium and diluted with buffer or purified water during the staining process. Leishman stain is a differential stain that is used to stain the different elements of the cells in different ways. After two minutes, the slide is flooded with dye, then twice the amount of purified or buffer water is applied and thoroughly combined with the stain. This is set aside for 5–7 minutes. After that, the prepared peripheral smear is stained. For staining purposes, Leishman stain is used. Methylene blue and Eosin dye are mixed in an alcohol medium and diluted with buffer or purified water during the staining process. Leishman stain is a differential stain that is used to stain the different elements of the cells in different ways. After two minutes, the slide is flooded with dye, then twice the amount of purified or buffer water is applied and thoroughly combined with the stain. This is set aside for 5–7 minutes. The nucleus of a typical lymphocyte is about 8 m in diameter. Divide the number of cells that cross the diameter of the field of view by the diameter of the field of view to get the length of one cell [7].

Calculation of MCV The mean corpuscular value may be measured manually or with the aid of a computer. It is determined using the manual method by multiplying the haematocrit value by 10 and dividing it by the amount of RBC. MCV = Hct (%) ×10/RBC x1012/l. In healthy people, the MCV ranges from 84 to 96 fl. The MCV value is measured using an electronic computer for this study. Automatic analyzers may be used to calculate the mean corpuscular meaning in a variety of ways. A Coulter counter is an electronic blood cell counter that measures volume. The red cells move through a narrow aperture one by one and produce a signal that is proportional to their volume. Other digital counters use tools that measure refracted, diffracted, or dispersed light to determine red blood cell amount.

Result

Table 1: Categories of RBC size ranges and its MCV range

Categories	RBC size range	MCV value range
I	6-7.0micrometer	71fL-95fL
II	7-7.6 micrometer	76fL-91fL
III	8-8.4 micrometer	82fL-85fL

Table 2: Categories of Mean RBC size and MCV value

Categories	RBC size	MCV Value
I	6.6	82 fL
II	7.3	83 fL
III	8.2	84 fL

Table 3: Statistical Analysis (Linear Regression)

	Coefficient	Std. Error	t	P
Constant	74.4	17.1	4.2	<0.001
RBC size(X)	1.1	2.4	0.5	0.6
MCV value(Y) = 74.374 + (1.165 * RBC size(X))				

Discussion

According to the results of the analysis, the samples were grouped into three groups based on the scale of the RBCs. Category I samples have RBC sizes of 6- 7 micrometres, Category II samples have RBC sizes of 7-7.6 micrometres, and Category III samples have RBC sizes of 8-8.4 micrometres. The MCV value's Range and Mean are observed and statistically compared with each subclass. The observations are discussed in the next segment. RBC size and MCV values are reported for the blood sample obtained. The scale of the erythrocytes in the sample blood is classified into three groups, each with a mean value.

The first type, as seen in a micrometre scale of 6-7.0. The scale ranges from 7-7.6 micrometres in the second group. The scale limit for the third category is 8-8.4 micrometres. According to Mary Louise *et al.* [8] the standard scale of human RBCs is between 6.2-8.2 micrometres. The same RBC size spectrum was seen in our sample, ranging from 6.0 to 8.4 micrometres. This is in line with the findings of Mary *et al*.

The MCV's mean and distribution are noted separately for each of the three divisions. The MCV value varies from 71Fl to 95Fl for RBCs with a size limit of 6 to 7.0 micrometres. The MCV value ranges from 76Fl to 91Fl for RBCs with a size limit of 7 to 7.6 micrometres, and from 82.4fL to 85fL for RBCs with a size limit of 8 to 8.4 micrometres. According to Sarma PR [13], the usual MCV value is between 80 and 94fl. The MCV found in this sample ranges from 70 to 94 feet. The normal lower limit of the MCV value in our sample is 70fl, and the normal upper limit of the MCV value in our study is 94fl, despite small variations in the values. This demonstrates that only the upper limit value corresponds to the Sarma analysis [9].

Mean RBC size for category I is 6.6 micrometre, with an MCV value of 82 fL, the mean RBC size for category II is 7.3 micrometre, with an MCV value of 83 fL, and the RBC size for category III is 8.2 micrometre, with an MCV value of 84 fL. In a non-statistical perspective, there is a relationship between RBC size and MCV value; as RBC size grows, so does MCV value. A statistical analysis is performed to determine if there is a meaningful relationship between RBC size and MCV importance, as well as the regression coefficient.

In the mathematical study, the regression coefficient of 1.1 for RBC size was discovered, indicating that a decrease in RBC size of 1 micrometre results in a change in MCV value of 1.1fL. Since the R2 on the graph is 0.016 (1.6%), it means that just 1.6 percent of the difference in MCV value is explained by RBC size alone, leaving the other 98 percent to be explained by other variables such as PCV value, RBC count, and so on. Finally, the P-value is 0.63, indicating that there is no statistically meaningful association between RBC size and MCV value.

By the statistical analysis, we find out that there is a relation between the RBC size and the MCV values. We can see that there is a connection between RBC size and MCV values using statistical analysis. When the size of the RBCs increases, the MCV increases as well. However, the mathematical p-value indicates that this improvement is not statistically important. In addition, our research discovered that a 1 micrometre increase in RBC size results in a 1.1 fl increase in MCV value. Despite the fact that the MCV is a measure of RBC size in the peripheral blood, it is only measured using the Packed Cell Volume (PCV) and the total RBC count, according to the formula provided by Wintrobe's *et al*. and Dacie *et al.* [14] MCV in fl= (PCV in percentage/RBC count x1012/L) x10. The full relationship between RBC size and MCV with a coefficient value is discovered in this analysis.

From this study we concluded that RBC histograms assist us in prediction of smear picture &correct interpretation of histogram gives better idea about etiopathological workup of various hematological conditions and save precious time of pathologist. From reading histograms, we can have better idea about what to expect when weactually evaluate the peripheral blood film by microscopy. The speed, accuracy and reliability of the modern analyzers allow us to analyze very large number of complete blood count analysis within very short period of time which is impossible in case of manual reporting. It gives us enough time to evaluate abnormal blood films, consider diagnostic clues and correlate clinical findings to histograms. They facilitate us to report all samples with confidence and efficiency and all of which increase the standard of patient health care.

Conclusion

According to the analysis, a pathologist will evaluate macrocytic or microcytic RBCs without using an automatic

analyzer and without scanning for and comparing the size of small lymphocytes by using the peripheral smear alone and comparing the MCV values. There is a need for a scale (in-built Vernier caliper) in the microscope, which is useful for RBC size measurement and also saves time. According to the findings, there is a statistically significant linear association between RBC size and MCV.

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