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Analysis and comparison of RBC size in peripheral smear and mean corpuscular volume in automated method of peripheral smear

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Abstract
Introduction: MCV test is done to measure the average size of the red blood cells and its abnormalities. It is a part of a regular check-up to find any abnormalities present. MCV is calculated by using haematocrit values and RBC count. Though the MCV is an indicator for the RBC size, the calculation only used the PCV and RBC count. This study is done to find out the relation between the MCV and RBC size.
Materials and Methods: Blood samples have been collected from a sample size of 50 patients randomly from a dental institutional laboratory. Anticoagulants are used to prevent the blood from clotting. The RBC size is calculated by using a standard formula from the peripheral smear of the obtained sample. MCV is obtained from the laboratory by using automated hemology analyser. Results are analysed statistically.
Results and Conclusion: As the study states the method of RBC size calculation by using the peripheral smear alone and compared to the MCV values, a pathologist can very well analyze the macrocytic RBCs or Microcytic RBCs without an automated analyzer and there is no need of searching and comparing the size of small lymphocytes. A need for scale (in-built Vernier caliper) in the microscope which is useful for the RBC size analysis and it is also a time-saving method. This study concludes that a linear relationship between the RBC size and MCV statistically.

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

Introduction
Red blood cells which are also called erythrocytes is a cellular component of the blood. These are millions in number present in the circulation of vertebrates which gives its characteristic color. A normal human RBC which is discocyte in shape is approximately 7.5 to 8.7 μm in diameter and 1.7 to 2.2 μm in thickness. The Haemoglobin molecules which are essential for the transport of gases within the body circulation are contained in the cytosol of RBC. The membrane regulates the volume of cytosol that is present in the intracellular RBC fluid and averages 94 μm3 at 300 mOsmol/kg (the standard unit osmol measures osmotic pressure as osmols per kilogram [Osmol/kg]; milliosmol [mOsmol] is one-thousandth of one Osmol) [1]. The membrane of the RBC cell is composed of a phospholipid bilayer and network of spectrin molecules which is two-dimensional underlying it. The compiled property of the spectrin molecules and phospholipid bilayer result in the discocyte morphology of healthy RBC. Elastic and bio rheological properties of the cell membrane are provided by it. The shear elastic properties of the RBC is provided by the spectrin network or cytoskeleton. Integral and peripheral proteins connect the bilayer and spectrin network. These connections involved in protein binding are referred to as vertical interactions; binding that is involved in the spectrin network formation which is two-dimensional are referred to as horizontal interactions [2].
The disruptions to these vertical and horizontal interactions tend to result in changes to the spectrin network density, which in turn invariably causes changes in cell morphological, membrane fluctuations, and RBC deformability for many RBC hereditary disorders. The RBC shows a unique ability for repeated large deformation. It is responsible for the movement of these cells through blood vessels during circulation which is as small as 2–3 μm in diameter. Dynamic cytoskeleton remodeling of the spectrin network was shown to facilitate this fluidity [3].

The biconcave shape and corresponding deformability of the human red blood cell (RBC) is an essential feature of its biological function [4]. The main function of the RBC and its hemoglobin is oxygen conduction from the gills or lungs to all the body tissues and to carry carbon dioxide, which is a waste product of metabolism from the body tissues to the lungs, where it is excreted out.

MCV stands for mean corpuscular volume. The three main corpuscles (cells) in the circulating blood are red blood cells, white blood cells, and platelets. Among these MCV tests are done to measure the average size of the red blood cells and its abnormalities. The mean corpuscular blood test is normally a part of complete blood count. It is a part of a regular check-up to find any abnormalities present. The abnormalities are used to find whether there is an occurrence of any diseases such as anemia which is a blood disorder, any vitamin deficiency, and any other medical condition. There is no particular preparation for the test. This test is performed by health care professionals, who are involved in taking a blood sample from a vein usually in the arm, using a small needle. After the needle is inserted, a small amount of blood will be collected into a test tube or vial. A little sting is felt during taking out blood because of the movement of the needle. This usually takes less than five minutes. The sterilization of the syringe is required to avoid any infection. Abnormality in mean corpuscular value may result in anemia mainly iron deficiency anemia and thalassemia. Anemia is a condition in which your blood has a lower than normal amount of red blood cells. Iron-deficiency anemia is the most common form of anemia. Thalassemia, an inherited disease that can cause severe anemia. The above-mentioned disorder is due to a decrease in mean corpuscular volume.

The increase in mean corpuscular volume, that is the increase in the size of RBC than the normal value may result in vitamin B12 deficiency, deficiency in folic acid, another type of B vitamin, Liver disease, Hypothyroidism. Diet, activity level, medicines, a women's menstrual cycle, and other considerations can affect the results. Inherited disorders of erythrocyte volume homeostasis are a heterogeneous group of rare disorders with phenotypes that ranges from dehydrated to overhydrated erythrocytes. The cellular volume homeostasis should be maintained and it is essential for the healthy survival of the erythrocyte [5]. Perturbation of this homeostasis is a common feature of several inherited anemia's which leads to abnormal RBC size. Several pathways mediate water and solute homeostasis in normal red cells, where cellular volume is primarily controlled via regulation of monovalent cation content [6]. The sodium-potassium ATPase pump (Na+K+ATPase) maintains the intracellular low sodium, high potassium composition of the erythrocyte by actively transporting sodium out of and potassium into the cell. RBC swells up when there is inward sodium leakage exceeds the potassium leak out and it shrinks when the potassium leaks out exceeds the inward sodium leak. Alterations in membrane permeability are detected by the analysis of intracellular potassium, sodium, and altered indices of erythrocyte hydration, and total cation content, e.g. increased or decreased mean corpuscular hemoglobin concentration (MCHC) [7].

Hereditary spherocytosis which is a rare blood disorder in which defects in the red blood cells cause them to be shaped like spheres and break down easily can also be identified with the help of a mean corpuscular volume test. An elevated mean corpuscular volume (MCV) is associated with aging, nutrition, alcohol abuse and more, and it is known as a survival predictor in chronically ill patients [8]. Normal production of RBC is dependent on the availability of the required "ingredients" (i.e., iron, folic acid, and vitamin B12), a normal functioning bone marrow, and erythropoietin for stimulation of red cell production. Thus, any deficiency of the above-mentioned factors also leads to a change in MCV value. This study is done to evaluate and compare the RBC size and mean corpuscular volume with its related abnormalities.

Materials and Method

Selection of sample and Blood collection method

A prospective study was conducted on forty dipotassium EDTA blood samples which were collected from randomly selected patients attending routine blood examination from Clinical Laboratory of a tertiary care dental hospital in South India, after obtaining their consent. The purpose of making a smear for prevention of sample from being lost while staining procedure. Precleaned slides are labelled with the patient's name (or other identifiers), date and time of collection. Blood films are made by placing a drop of blood on one end of a slide and using a spreader slide to disperse the blood over the slide's length. This monolayer of the blood sample is obtained. The drop is smeared lightly and quickly with a wedge technique to leave a thin "feather" edge where all cells may be examined individually, particularly red blood cells. The surface of the slide with smear should not be touched. The sample size of the blood collection about 50.

Calculation of RBC size

Subsequent staining for the prepared peripheral smear is done. Leishman stain is used for staining purposes. Leishman stain is a mixture of Methylene blue, and Eosin dye, prepared in Alcohol medium and diluted with buffer or distilled water during the staining procedure. Leishman stain is a differential stain that is used to variably stain the various components of the cells. The slide is flooded with stain and left for two minutes, after which twice the volume of distilled or buffer water is added and mixed well with the stain. This is left for five to seven minutes. Leishman stain is sensitive to any change in pH so that the water used in the preparation of the stain and the dilution must have a pH value of between 6.6 and 6.8 [9]. The prepared smear is then observed in the light microscope. The magnification used is 100X, to obtain accurate results.

Normal RBCs have a diameter of 6-8 μm. On a peripheral blood smear, normal RBCs are disc-shaped with a pale-staining central area called the central pallor. When judging red cell size on a blood smear, the classic rule of thumb is to compare them to the nucleus of a small normal lymphocyte. The normal lymphocyte nucleus has an approximate diameter of 8 μm. To figure the length of one cell, divide the number of cells that cross the diameter of the field of view into the diameter of the field of view [10].

$$\frac{\text{Diameter of field of view}}{\text{Estimated number of cells that cross the diameter}} = \text{Diameter of one cell}$$

MCV value calculation

Mean corpuscular value is calculated either manually or using an automated machine method. In the manual method, it is calculated by multiplying haematocrit value with 10 and dividing it by the number of RBC [11].

MCV = Hct (%) ×10/RBC x10^{12}/l

The normal value of the MCV in normal humans is 84-96 fl. For the present research, the MCV value is observed using an automated machine. The Mean corpuscular value can be determined in the number of ways using automatic analyzers. Coulter counter is a volume-sensitive automated blood cell counter. In this, the red cells pass one-by-one through a small aperture and generate a signal directly proportional to their volume. Other automated counters measure red blood cell volume through techniques that measure refracted, diffracted, or scattered light.

Result and discussion

From the study done, the samples are divided into three categories according to the RBC sizes. Category I is considered for the samples which shows the RBC size of 6-7 micrometre, Category II is considered for the samples with RBC size of 7.1-8.0 micrometre and the Category III is considered for the samples with RBC size 8.1-9 micrometre. For each category the Range and Mean of the MCV value is detected and compared statistically. The interpretation of the results is given below.

For the blood sample collected, RBC size and MCV values are noted. The erythrocyte size for the sample blood obtained is divided into three categories with its mean value. As shown in Table 1, the first category is of size 6.03-7.0 micrometre. The second category is of size 7.06-7.76 micrometres. The third category is of size limit 8.0-8.3 micrometres. The study done by Mary Louise *et al* [12] shows that the normal human RBC size is in a range of 6,2-8.2micrometre. Our study also shows the same RBC size range from 6.03 to 8.3 micrometres. This is well correlating with the Mary *et al* study.

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

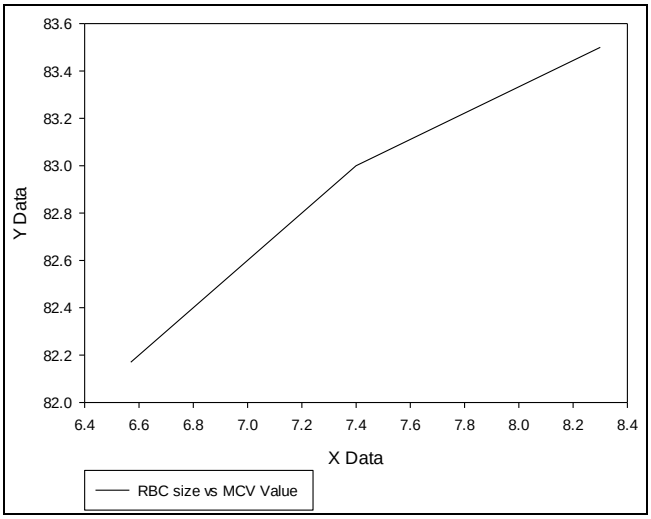
Categories	RBC size range	MCV value range
I	6.03-7.0micrometer	70fL-94fL
II	7.06-7.76 micrometer	75fL-90.7fL
III	8.0-8.3 micrometer	82.4fL-84.6fL

For all the three categories the mean and range of the MCV are noted separately. For the RBC size limit of 6.03 to 7.0 micrometres, the MCV value ranges from 70Fl to 94Fl. For the RBC size limit of 7.06 to 7.76 micrometres, the MCV value ranges from 75Fl to 90.7Fl, and for the RBC size limit of 8 to 8.3 micrometres, MCV value ranges from 82.4fL to 84.6fL. Sarma PR [13] mentioned that the normal MCV value is between 80 to 94fl. In this study, the range of the MCV found is from 70fl to 94fl. Though the values are in a minor variation the normal lower limit of the MCV value in our study is 70fl and the higher limit of the MCV in our study is 94fl. This shows that only the higher limit value is correlating with the Sarma study.

Table 2 shows that for the category I the mean RBC size is 6.53micrometre for that the MCV value is 82.17 fL, the mean RBC size of the category II is 7.4 micrometre and the mean MCV for that is 84 fL and for the category III, the RBC size is 8.3 micrometre and the mean MCV value is 83.5 fl. With a non-statistical view, there is a relation between the RBC size and MCV value, as the RBC size increases, the MCV Value also increased (Graph 1). Statistical analysis is done to find out whether any significant relation between the RBC size and the MCV value and also to find out the regression coefficient.

Table 2: Categories of Mean RBC size and MCV value

Categories	RBC size	MCV Value
I	6.57	82.17
II	7.4	83
III	8.3	83.5



Graph 1: RBC size(X) with MCV value(Y) Regression analysis

As Table 3 shows the regression coefficient of 1.165 for the RBC size is found out in the statistical analysis, which indicates that a change in the RBC size of 1 micrometre results in a change in MCV value by 1.165fL. As the R2 is 0.016 (1.6%) found on the analysis, which indicates that 1.6% of the variation in MCV value is explained by the RBC size alone, the remaining 98% might have contributed by some other variables like PCV value, RBC count, etc. Finally, the P-value is calculated as 0.63, so there is no significant correlation between the RBC size and the MCV value statistically.

Table 3: Statistical Analysis (Linear Regression)

	Coefficient	Std. Error	t	P
Constant	74.374	17.088	4.352	<0.001
RBC size(X)	1.165	2.395	0.486	0.634
MCV value(Y) = 74.374 + (1.165 * RBC size(X))				

By the statistical analysis, we find out that there is a relation between the RBC size and the MCV values. Whenever there is an increase in the RBC size, the MCV also increases. But according to the statistical p-value, this increase is not statistically significant. Also, our study finds out that an

increase in the RBC size of 1 micrometre, there is an increase in MCV value by 1.165 fl. Though the MCV is an indicator of RBC size in the peripheral blood, it is calculated only by using the Packed Cell Volume (PCV) and the total RBC count with the formula given by Wintrobe's *et al* and Dacie *et al* [14] MCV in fl= (PCV in percentage/RBC count x1012/L) x10. In this study, we find out the complete relationship between the RBC size and the MCV with a coefficient value.

Conclusion

As the study states the method of RBC size calculation by using the peripheral smear alone and compared to the MCV values, a pathologist can very well analyze the macrocytic RBCs or Microcytic RBCs without an automated analyzer and there is no need of searching and comparing the size of small lymphocytes. A need for scale (in-built Vernier caliper) in the microscope which is useful for the RBC size analysis and it is also a time-saving method. This study concludes that a linear relationship between the RBC size and MCV statistically.

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