Comparison of Morphological Analysis of RBC through Peripheral Smear and Automated Method

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Abstract
Aim: To compare the morphology of red blood cells by peripheral smear and automated method.
Objective: Comparison of morphology of red blood cells by using peripheral smear and automated method.
Background: Counting of red blood cells (RBC) in blood cell images is very important to detect as well as to follow the process of treatment of many diseases like anaemia, leukaemia etc. However, locating, identifying and counting of red blood cells manually are tedious and time-consuming that could be simplified by means of automatic analysis.
Reason: To know which is the most effective method of analysing morphology of RBC.
Keywords: Red blood cells; Observers; Automated method

Introduction
Red blood cells (RBCs), also known as erythrocytes, are the most common type of blood cells in our body and it is the principal means of delivering oxygen to the body tissues. The red blood cells are typically biconcave disks: flat and depressed in the centre, with a dumbbell-shaped cross section, and a rim on the edge with the torus shaped disk [1]. Red blood cells in mammals are unique amongst vertebrates as they do not have nuclei when mature. Anaemia is the lack of red blood cells and/or haemoglobin. This results in a reduced ability of blood to transfer oxygen to the tissues [2]. Normal, mature RBCs are biconcave, disc-shaped, a nuclear cells measuring approximately 7-8 microns in diameter on a peripheral blood smear with an internal volume (MCV) of 80-100 femto liters (fL). The term used to describe RBCs of normal size is “normocytic”. Anaemia is classified by the size of the red blood cells which is either done automatically or on microscopic examination of a peripheral blood smear. The size is reflected in the mean corpuscular volume (MCV). If the cells are smaller than normal (under 80fL), the anaemia is said to be microcytic; if they are normal size (80-100fL), normocytic; and if they are larger than normal (over 100fL), the anaemia is classified as macrocytic [3]. MCV measures only average cell volume. The MCV can be normal while the individual red cells of the population vary wildly in volume from one to the next. Such an abnormal variation in cell volume is called anisocytosis [1]. The degree of anisocytosis in a sample of blood is known as the red cell distribution width (RDW).

Materials and Methods
The study was done using 50 blood smears of patients with different age groups.

Blood samples
All venous blood specimens were collected in tubes containing ethylenediaminetetraacetic acid (K3EDTA) and then were analysed.

Automated method
After thorough mixing of each blood sample on an automated mixer for 3-5 min, a mean corpuscular value was obtained in which value between 80-100 were considered normal.

Manual method
Thin air-dried blood smears made after thorough mixing of each sample were stained manually with leishman’s stain and examined under light microscopy with a X100 oil-immersion lens.

Results and Discussion
Manual method was done with two different observers (pathologists) and the results obtained were compared with the results obtained from automated method. The similarities among the results are shown in the graph shown in Figure 1. Out of 50, 38% of the results were same with both the observers and
the automated method. 24% of the results were same between 2 observers 17% of the results were common between one observer and the automated value. Only 4% of the results were different among all the observers and the automated method. Among all 50 slides when examined by the observer1, 24 slides were normocytic, in case of observer 2, 39 were normocytic and in automated method 26 were normocytic. Most cases of normocytic anemia are caused by blood loss, suppressed production of RBCs, or hemolysis [2]. Macrocytic cells were seen in 9 slides by observer 1, only one slide had macrocytic cells by observer 2 and 3 slides in automated method. Macrocytic is usually seen to differentiate between megaloblastic and nonmegaloblastic causes. Megaloblastosis is seen with and folate deficiency, MDS and CDA, HIV infection, and rare inborn errors of metabolism, while nonmegaloblastic causes include liver and thyroid disease, alcohol, Down syndrome, aplastic anemia, and reticulocytosis. Medications can be responsible for both megaloblastic and non-megaloblastic anemia, while RBC agglutination may lead to spurious macrocytosis [2]. Microcytic cells are seen in 17 slides by observer 1,10 slides had microcytic cells by observer 2 and 20 slides were microcytic in automated method. In classic cases, the morphological differentiation of the three common microcytic anemias is straightforward [4].

Figure 1: Manual method was done with two different observers (pathologists) and the results obtained were compared with the results obtained from automated method.

Conclusion

The review of red blood cell morphology is the most important step in the evaluation of a patient with anemia. It can be very useful in evaluating microcytic, normocytic, and macrocytic anemias and is especially helpful in the patients with hemolysis [5]. It can be concluded that for diagnostic purposes results obtained from two different observers or results from both the observers and the automated value can be considered.

References

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