Original Research Article

Comparison of peripheral blood smear and automated cell counter in 100 cases of anemia

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Abstract

Background: Hemogram/ Complete Blood Count (CBC), performed on hematology analyzers, is one of the most commonly ordered blood test in clinical practice especially in patients presenting with fever. The aim of the present study was to compare accuracy of RBC histograms shown by our 5 part cell counter to that of microscopy examination of blood film stain by field stain.

Materials and methods: The present observational study was conducted during June 2020 - August 2020. Samples were run on 5 part hematology analyzer. Population of both anemic and healthy individuals in the age group 0-60 years were evaluated for the study. Total 100 patients in the age group of 0- 60 years were enrolled in this study. Hematological parameters were obtained by using 5 part automated hematology cell counter. The hemoglobin concentration and red blood cell (RBC) indices, encompassed mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and erythrocyte count.

Results: We studied 100 blood samples of patients. Among them 82 cases showed correlation between histogram to that of the peripheral blood smear examination.
Conclusion: The result of the study shows that the automated hematology analyzer are appropriate for screening purposes because it increases the turnaround time and reduces the labor cost. But to diagnose and differentiate different types of anemia manual scan of peripheral smear is a method of choice.

Key words
Peripheral blood smear, Automate cell counter, Anemia, Comparison.

Introduction
Hemogram/ Complete Blood Count (CBC), performed on hematology analyzers, is one the most commonly ordered blood test in clinical practice especially in patients presenting with fever. Ever since Wallace H. Coulter discovered method for counting particles in fluid and patented in 1953, technical up gradations of automated Hematology Analyzers have made them extremely sophisticated, fast and intelligent [1, 2]. Many articles have been published showing very wide sensitivity and specificity depending upon methodology in various hematology analyzers. Peripheral blood smear examination has been an important part of investigation for various hematological disorders since decades and also major diagnostic tool especially for etiopathological work up of different hematological disorder. The automated hematology analyzer has replaced the traditional manual methods for measuring various haematological parameters as the initial screening method in most of the hospital nowadays [3]. This study is an attempt to standardize few automated red cell parameters and to compare these with microscopic examination of peripheral blood smear. Automated cell counter provide histogram of RBCs which give us important clue regarding particle size, volume. This RBCs histogram if interpreted along with other important RBCs indices like Red cell distribution width (RDW) and mean corpuscular volume (MCV), have been found very useful in work up of many hematological disorders and may provide major diagnostic clue in condition like anemia, thalassemia [4-7]. The aim of the present study was to compare accuracy of RBC histograms shown by our 5 part cell counter to that of microscopy examination of blood film stain by field stain.

Materials and methods
The present observational study was conducted during June 2020 - August 2020. Samples were run on 5 part hematology analyzer. Population of both anemic and healthy individuals in the age group 0-60 years were evaluated for the study.

Sample size
Total 100 patients in the age group of 0-60 years were enrolled in this study.

Collection of blood sample
3 ml of blood samples were collected aseptically by standard phlebotomy technique by trained phlebotomist from each subject into tri-potassium Ethylenediamine tetra-acetic acid (K3 EDTA) anticoagulant.

Screening of blood samples
Hematological parameters were obtained by using 5 part automated hematology cell counter. The hemoglobin concentration and red blood cell (RBC) indices, encompassed mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and erythrocyte count.

Results
We studied 100 blood samples of patients. Among them 82 cases showed correlation between histogram to that of the peripheral blood smear examination. Distribution of such cases is shown in Table - 1. Out of 100 samples total 18 cases did not show correlation between histogram to that of peripheral blood smear.
examination. Distribution of such cases is shown in Table - 2.

**Table – 1:** Distribution of cases which showed correlation.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Total no. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Deficiency Anemia</td>
<td>31</td>
<td>37.82</td>
</tr>
<tr>
<td>Megaloblastic anemia</td>
<td>12</td>
<td>14.63</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>07</td>
<td>8.53</td>
</tr>
<tr>
<td>High reticulocytes count</td>
<td>08</td>
<td>9.75</td>
</tr>
<tr>
<td>Post Iron deficiency anemia therapy</td>
<td>08</td>
<td>9.75</td>
</tr>
<tr>
<td>Beta Thalassemia major</td>
<td>09</td>
<td>10.97</td>
</tr>
<tr>
<td>Beta Thalassemia minor</td>
<td>07</td>
<td>8.54</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table – 2:** Distribution of cases which did not show correlation.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Total no. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets clumps</td>
<td>3</td>
<td>16.67</td>
</tr>
<tr>
<td>Giant platelets</td>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td>High leucocytes count</td>
<td>6</td>
<td>33.32</td>
</tr>
<tr>
<td>Cold agglutinations</td>
<td>1</td>
<td>5.56</td>
</tr>
<tr>
<td>High Nucleated RBCs</td>
<td>3</td>
<td>16.67</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>1</td>
<td>5.56</td>
</tr>
<tr>
<td>Post transfusion cases</td>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>

Interpretation of histograms, in conjunction with the numerical data can be clinically useful in the diagnosis and follow up of many hematological and non-hematological conditions. We have to look after shape, centre, and spread of histogram along with overall pattern for correct reading of it. This pattern should be read along with other reference normal curve and/or to be confirmed microscopically by expert. Symmetric and skewed shapes were observed in RBC histogram which were seen and easily identified but others variation in shape may be more challenging, especially when two populations of red cells are present and such cases should be confirmed by microscopy. Direct inspection of the distribution curve gives us information about size of red blood cells and their variation microcytes and macrocytes, however the estimation of the number of cells from the histogram should be avoided as erroneous results can arise because the frequency curve shows only the relative information and not give us the actual number of cells in each size range [8].

**Discussion**

Number of factors like red cell agglutination, alteration in red cell shape, and inclusion of leukocytes, inclusion in red blood cells like parasite infection, Hb H inclusion may affect the histogram. These factors, in one way or another, influence the histogram’s appearances and accordingly will have a erroneous effect on histogram [9]. To reduce the effect of these problems, every manufacturers design their instruments and reagent systems to specifically prevent and correct for interferences. They develop mathematical algorithms for particle counting and produce numeric data, graphic data, scatter plots, and interpretative comments that will assist or alert the users to potential incorrect results [10, 11].

The discrepancy between automation and manual scan of peripheral blood in the measurement of hemoglobin and red blood count may result in misclassification for the diagnosis of anemia. This signified that the manual microscopic method still has some advantages over the automated method [12].

In addition our study revealed statistically 82 subjects out of 100, correlated positively when both automated and manual methods were compared. This manifests that the automated analyzer readings correlated well with the manual methods. The results of the present study are in contrast with an earlier report by Pierre and Novis, et al. [13, 14] who reported that automated hematology analyzer are more accurate in the detection of specimens with morphological abnormality than the traditional eye count method. Whereas, (Table – 2) 18 cases would have discordance findings and contributed
to some statistical variations observed between hematology analyzer and manual scan of peripheral blood smear adopted in our study. De Gruchy, also affirmed that the manual scan of peripheral blood smear which may provide important diagnostic help in patients with normal blood counts are frequently overlooked, his results illustrated that the four non-anemic patients reported had normal blood counts. In a blood smear of each he found important morphological abnormalities. In each case the findings came as a surprise to the clinician and led to a diagnosis which he had not formerly suspected. In most automated laboratories, manual microscopic examination are essential to the cases for which instrument ‘flags’, indicates the potential presence of cells not reliably identifiable by automated methods or findings that may interfere with automated analysis (such as overlap in the distribution of different cell types or interference from matrix components such as cryoglobulin) [13] Similarly, Ike et al.[12] also support our study and suggested that microscopic examination should always be used to validate the automated results.

**Conclusion**

The automated hematology analyzer readings are as reliable as the standard manual method, even though the manual scan of peripheral smear provides additional diagnostic information. The result of the study shows that the automated hematology analyzer are appropriate for screening purposes because it increases the turnaround time and reduces the labor cost. But to diagnose and differentiate different types of anemia manual scan of peripheral smear is a method of choice.

**References**

12. Ike S.O., Nubila, Ukaejiofo E.O., et al. Comparison of haematological parameters determined by the
